

PB-216 847

BENTHONIC INVERTEBRATES AS AN INDEX OF  
WATER QUALITY

Theodore A. Olson, et al

Minnesota University  
Minneapolis, Minnesota

September 1964

DISTRIBUTED BY:

**NTIS**

National Technical Information Service  
U. S. DEPARTMENT OF COMMERCE  
5285 Port Royal Road, Springfield Va. 22151

September 1962 - September 1964

Progress Report

PHS Research Grant WP-320

Benthonic Invertebrates as an Index of Water Quality

Prepared by

Theodore A. Olson, Principal Investigator  
Myrtle E. Rueger, Research Fellow

Reproduced by  
NATIONAL TECHNICAL  
INFORMATION SERVICE  
U S Department of Commerce  
Springfield VA 22151

Details of illustrations in  
this document may be better  
studied on microfiche

School of Public Health  
University of Minnesota

September 1964

## CONTENTS

Page

### I. GENERAL SCOPE OF THIS INVESTIGATION

|                                                            |    |
|------------------------------------------------------------|----|
| A. INTRODUCTION.....                                       | 1  |
| B. GENERAL SCOPE AND OBJECTIVES OF THIS INVESTIGATION..... | 2  |
| C. AVAILABLE METHODS OF RESPIROMETRY                       |    |
| 1. Introduction.....                                       | 3  |
| 2. Chemical Methods.....                                   | 3  |
| 3. Electrical Methods.....                                 | 4  |
| 4. Electro-Chemical Methods.....                           | 5  |
| 5. Manometric Methods.....                                 | 8  |
| 6. Volumetric Methods.....                                 | 10 |
| D. TABLE.....                                              | 11 |
| E. BIBLIOGRAPHY.....                                       | 14 |

### II. ELECTRO-CHEMICAL METHOD AS USED IN THIS INVESTIGATION

|                                |    |
|--------------------------------|----|
| A. OBJECTIVES.....             | 19 |
| B. PROCEDURE.....              | 19 |
| C. RESULTS.....                | 20 |
| D. DISCUSSION AND SUMMARY..... | 22 |
| E. BIBLIOGRAPHY.....           | 23 |
| F. PLATES AND FIGURES.....     | 25 |

### III. MANOMETRIC METHOD AS USED IN THIS INVESTIGATION

|                                |    |
|--------------------------------|----|
| A. OBJECTIVES.....             | 47 |
| B. METHODS AND PROCEDURE.....  | 47 |
| C. RESULTS AND DISCUSSION..... | 49 |
| D. SUMMARY.....                | 52 |
| E. TABLES AND FIGURES.....     | 53 |
| F. BIBLIOGRAPHY.....           | 71 |

## I. GENERAL SCOPE OF THIS INVESTIGATION

### A. INTRODUCTION

Biological analysis has served to verify, complete, and broaden chemical, physical, and bacteriological findings in drinking water and sewage. The community of animals in a stream or body of water reflects the average conditions and avoids the limitations involved in chemical, bacteriological, or physical standards, which give only "spot" results. With continuous monitoring of waters, conditions can be determined much better than has been possible in the past with spot analysis; however, unless probes are placed in all types of conditions, results may not give a true picture of all parts of the body of water. Aquatic insects, with a span of life in water of weeks to several years, constitute a valuable index of the average quality of water.

It has been pointed out by the Principal Investigator (1957), Gaufin and Tarzwell (1952, 1956), and Hyne (1960) that, to make the fullest use of biological indicators in pollution studies, one should deal with the complex of bottom-dwelling organisms in groups according to their morphological adaptations and physiological requirements rather than with a specific index species. In connection with this, there is a great need for additional knowledge concerning the reactions of the individuals which make up the ecological units. Such information would make judgments concerning the associated environment more satisfactory and would increase the dependability of this type of data. For example, one might be able to estimate more accurately the minimum concentrations of oxygen which have existed in a specific stream or body of water during a given preceding period if the oxygen requirements of each member of the biocoenose were better known.

The Kolkwitz and Marsson Saprobien system (Kolkwitz and Marsson, 1908, 1909, and Kolkwitz 1950) lists organisms in relation to their response to various degrees of pollution. This system has been modified by Liebmann (1951, 1960) and by American workers; however, it is apparent that there is a void of specific information relative to the exact requirements of the organisms listed, whether we consider them as individuals or as units of an ecological complex. As pointed out by Gaufin and Tarzwell (1956), careful comparison of these different lists will show that there is an appreciable lack of agreement as to the true station of many of the organisms. Usinger (1956) brings up another disturbing fact: that the Surber Square Foot Sampler which has been used in most surveys of stream-bottom organisms, does not give statistically significant data for total weights and numbers of organisms even when used in a relatively uniform riffle. To obtain reliable figures at the 95 per cent level of significance, 194 and 73 samples respectively, would be required; therefore, all existing data on stream-bottom organisms are statistically inadequate even though it may be the best that can be obtained because of the extreme variability of stream and other water habitats.

Oxygen consumption studies have been done on relatively few species of aquatic invertebrates; a list of the oxygen consumption of these organisms as related to their index organism classification is not available. With more and more emphasis being placed on water quality and on techniques which may be useful in assessing the characteristics of water, the value of biological indicators gains in importance. Studies which will clarify and improve our understanding of the index-organisms will be of cosmopolitan interest and wide practical use.

## B. GENERAL SCOPE AND OBJECTIVES OF THIS INVESTIGATION

The long term goal of this project is the evaluation of the ecology of benthic invertebrates which may be useful as indicators of water quality or as indicator organisms in bioassay studies. The original research was planned as a project of at least five years duration, during which time the various activities could be integrated. The primary research project was approved for a period of three years; this necessitated certain modifications of the original research plan.

One of the primary objectives of the proposed study was the determination of the individual oxygen consumption rates of common benthic invertebrates. The ultimate aim was to study representatives of all the important bottom-dwelling forms in an attempt to evaluate the oxygen requirements of each. Although this is a time-consuming process, it should yield information which can be used in the preparation of a list which indicates the requirements of each group or each species. Such a comparison of organisms should be useful in decisions involving their status as members of an index community.

It was decided that the Warburg apparatus would be used to make preliminary respiratory studies on aquatic invertebrates. This method has been used over a period of many years in the field of respiratory studies and is of sufficient sensitivity to measure the oxygen consumption of single specimens of most index organisms. It was envisioned that this procedure would be supplemented and perhaps supplanted by a flow-through method utilizing an electro-chemical oxygen sensing device and recorder since this method more nearly simulates natural conditions in a stream. This method would provide a continuous visual record of the respiration and enable study of the effect of water movement.

By using the same individuals or members of the same species in the Warburg apparatus and the flow-through electro-chemical system, a comparison would be made of the two systems. Since the electro-chemical method of oxygen determination is one of great promise and one that is currently practical, it was felt that a comparison would be useful and necessary. Our literature search has revealed no such comparison.

Utilizing the basic techniques of the Warburg and flow-through methods, it was planned that observations would be made on groups of organisms in contrast to single organisms. It was thought that this procedure might provide information on the nature and importance of interactions between individuals and species. This would represent a situation in which several forms may be in direct competition for the available oxygen and would simulate conditions in a stream or body of water.

It seemed desirable that some of the studies might be directed to the oxygen requirements of organisms held in special containers in their natural habitat. Also, it was planned that experiments would be carried out involving the transfer of organisms from one stream to another or from the laboratory to a stream in order to obtain information on the importance of conditioning and genetic differences.

## C. AVAILABLE METHODS OF RESPIROMETRY

### 1. Introduction

The technique of respiration experiments on aquatic animals must be based on the properties of the respiratory gases when dissolved in water or the suspending medium, such as physiological saline. Water in contact with the air becomes saturated with oxygen. For pure water in equilibrium with the atmosphere, the amount of gas dissolved will depend upon the barometric pressure and the temperature of the water. Temperature is the more important factor in most cases, for the amount of oxygen dissolved varies greatly with this parameter. Distilled water at 0 C and 760 mm pressure holds, when saturated, 14.6 mg of oxygen per liter; at 20 C it holds 9.2 mg (Standard Methods, 1960).

A difficulty and source of error in experiments on aquatic respiration is the presence of bacteria. It is generally impracticable to make experiments under aseptic conditions; therefore, it is necessary to limit the duration of the experiments except at very low temperatures so that the bacteria do not multiply to an extent which might seriously influence the results. When information on the normal metabolism of an animal is involved, a method must be selected that does not falsify the results by submitting the animal to grossly unnatural conditions.

Various methods and types of apparatus originally intended for other purposes have been modified for respiration studies on aquatic invertebrates. In some cases, these respirometers have been designed for specific studies and have been used only in the laboratory of their origin; they have not been commercially available. Although some of these are particularly suited to specific needs, they have limitations as well as inherent virtues. An organism does not undergo the same treatment in each; results from various methods will thus differ. Available methods for aquatic respiratory studies include the following types: (a) chemical, (b) electrical, (c) electrochemical, (d) manometric, and (e) volumetric. Each is discussed separately. Table 1 lists studies done by investigators using the various methods for determining oxygen consumption of immature aquatic insects. Studies done on aquatic crustacea have been summarized by Wolvekamp and Waterman (1960) and will not be repeated here.

### 2. Chemical Methods

One of the most widely used of this type has been the Winkler method introduced in 1888. Samples of water surrounding the animal are analyzed. Manganous chloride or sulfate is added to the water sample with strong alkali and potassium iodide. Manganous hydroxide immediately precipitates and promptly combines with the dissolved oxygen to form manganic hydroxide. Acidification dissolves the hydroxide and the quantity of iodine which is set free is equivalent to the oxygen originally present in the sample. Each atom of oxygen liberates one molecule of iodine. The iodine is then titrated with a standard solution of sodium thiosulfate using starch as an indicator. The concentration of oxygen is calculated from this obtained value; 1 cc of normal sodium thiosulfate corresponds to 8 mg of oxygen, or 5.6 cc of oxygen at normal temperature and pressure (Fox and Wingfield, 1938). Ordinarily the Winkler method is used for samples of water of about 200 cc in amount, but with special apparatus it has been successfully employed for samples as small as 1 cc or less (van Dam, 1935; Fox and Wingfield, 1938).

In this method, the carbon dioxide given off by the experimental animal is retained in the water. Allee and Oesting (1934) in their critical examination of the Winkler method for determining dissolved oxygen in respiratory studies with aquatic animals concluded that this method was sufficiently accurate if adequately handled. According to them, standard practice in such studies should include: (a) demonstration that there is no iodine absorption or adsorption; (b) the sample should have less than 0.005 mg of nitrite nitrogen per liter; and (c) samples should be free of ferrous iron. If these conditions are not satisfied, modified methods should be used. With the Winkler method, great care must be exercised in analyzing water with low oxygen content; otherwise oxygen from the air may enter the sample. Collecting the water sample under several centimeters of the best quality saturated-hydrocarbon mineral oil was recommended by Keys (1930).

A colorimetric method for determining the concentration of oxygen dissolved in water depends on the change in color of diaminophenol dihydrochloride at various concentrations of oxygen (Brinkman and van Schreve, 1942). The colorimetric estimation was made with a photoelectric colorimeter. In their opinion, it was much simpler than the macro- and micro-Winkler procedure and a double determination could be done on 0.6 ml of water.

### 3. Electrical Method

In this method two equal portions of gas pass through copper tubes in the center of which a heated platinum wire is fixed, forming a Wheatstone bridge (Ledig and Lyman, 1927; Punt, 1950-52). When the wires are surrounded by a gas, heat is conducted from them through the gas. Since the thermal conductivity of various gases and mixtures differ widely (Weaver, Palmer, Frantz, Ledig, and Pickering, 1920), changes in the composition of the gases around one wire is attended by a proportional change in the quantity of heat conducted by the gas from the wire and by a corresponding change in its temperature and electrical resistance. The difference between the two settings of the slide wire required to bring the Wheatstone bridge to balance, is a measure of the change in the composition which surrounds the first wire (Ledig and Lyman, 1927). The response of the galvanometer in the Wheatstone bridge circuit can be calibrated with a known composition of gas; other constituents must remain substantially constant (Punt, 1950-52). The galvanometer response can be photographed, permitting the registration of each respiratory movement. If CO<sub>2</sub> is being measured one portion of the gas is freed from CO<sub>2</sub> in absorbers, while the other remains unaltered.

This method has some advantages over other methods such as the Haldane since continuous records can be made, but it is accompanied by such limitations as the high cost of the equipment and the need for the services of a skilled worker familiar with electrical measurements (Ledig and Lyman, 1927). Different types of CO<sub>2</sub> output were observed with this method by Punt (1950-52) in his studies on adult Coleoptera, nymphal Orthoptera, pupal Lepidoptera, and nymphal Hemiptera. In some there was one outburst of CO<sub>2</sub> in about 10 minutes, and in others continuous CO<sub>2</sub> production. Levine, Warren, Tsivoglou, and Walker (1956) used a Gow-Mac Improved Standard differential thermal conductivity unit for the gas analysis in a prototype continuous oxygen analyzer they developed at the Robert A. Taft Sanitary Engineering Center.

Another method which could be classified as electro-physical involves stripping the oxygen from solution with nitrogen, following which the gaseous oxygen is measured on the basis of its paramagnetic properties. There are also methods in which the water being tested is passed through a column of thallium which is inert to the water molecule itself but, reacts to the oxygen which is dissolved to form thallic hydroxide. The water should be of low ionic strength. This thallic hydroxide can then be measured by means of an increase in electrical conductivity; since it is directly related to the amount of dissolved oxygen, we have a method for estimating the exact dissolved oxygen content of the water. Actually the latter device is so sensitive that oxygen may be evaluated in terms of parts per billion. If a radioactive thallium is used in the type of column just described, and a device is used which can measure the disintegrations of this material, one can extend the sensitivity to as little as one part of oxygen in a trillion.

#### 4. Electro-Chemical Methods

##### a. Principle

These methods for the determination of oxygen rely on a measurement of the current flowing in an external circuit of a cell. The potential of the cathode is usually in the area of 0.6 to 1.5 volts, and the current flowing is oxygen-limited. We thus have a means for the direct measurement of the dissolved oxygen concentration of the water in which the electrode, or transducer, is immersed. Within the electrochemical group there are two kinds of instruments. One is the galvanic cell type and the other is the polarographic cell type. These differ simply in the method by which the necessary cathode potential is obtained. In both instances, the anode must be nonpolarizable, or stated in another way, its electrode potential must remain essentially constant when the small amount of current produced by the oxygen reduction flows through the circuit. In the polarographic cell an external source, such as a battery, is used to apply voltage to obtain the necessary potential at the cathode. The galvanic cell, on the other hand, has a nonpolarizable anode reaction which generates the necessary potential for the cathode (which is easily polarized). This is possible because the anode is continuously and rapidly undergoing oxidation.

To make these cells useful as oxygen sensors and to eliminate interferences which can affect the limiting current, the proponents and developers of the oxygen electrodes have utilized plastic membranes to separate the cathode and anode from the water medium. This also permits a separation of the electrolyte from the measured stream of water which is passing the electrode. Hence, the construction of a transducer is as follows: The electrodes of the polarographic cell, for example, are generally made up of an inert substance with a high oxygen overvoltage; platinum is a good material. The anode in such a cell, with a constant voltage applied to the platinum cathode, is usually silver silver-oxide or silver silver-chloride. These electrodes are then surrounded by electrolytes, and separated from the stream of water to be analyzed by a polyethylene or teflon film. The current flows through the external circuit of the cell because voltage of the proper amount has been applied to the cathode to reduce oxygen. This oxygen reduction generates hydroxyl ions and these in turn diffuse into the electrolyte. At the anode, silver

oxide is plated on the electrode to close the circuit of the cell. The oxygen arrives at the cathode by diffusion from the membrane, having passed across the membrane and through the electrolyte to the cathode. A concentration gradient is therefore set up from the measured stream of water on the outside of the membrane to the cathode surface itself. In this way, the current which flows through the external circuit is a measure of the dissolved oxygen. Some of the limitations which are associated with this type of transducer are a high reaction to changes in the rate of flow of the water being tested and a sensitivity to changes in temperature. Both of these affect the diffusion rates across the plastic membrane.

The galvanic cell, which is another type of electrochemical transducer can be used both with and without plastic membranes. However, it is usually best to utilize the membrane inasmuch as it prevents lime deposits and excludes interfering substances. The galvanic cell has a nonpolarizable anode reaction which by itself generates the necessary potential for the easily polarized cathode. This it does by rapidly and continuously undergoing oxidation. A number of different cell designs have been utilized employing different kinds of anodes for this purpose. The anode reaction, being completely nonpolarizable, is spontaneous and generates an electron current which flows through the external circuit to the cathode. These galvanic cells, then, are entirely similar to the polarographic cells except for the method of voltage generation. The Okun galvanic cell (Mancy, Okun, and Reilley, 1962) is a good example of this type of transducer; it has been tested by various investigators during recent years and has been applied successfully to a number of practical situations.

#### b. Polarographic Method: Background

The dropping mercury electrode and the theory of the polarographic method of chemical analysis as developed by Professor Jaroslav Heyrovsky (1941) established the basic principles from which all the current electro-chemical oxygen transducers are derived. The essence of polarographic analysis is, to borrow a description from Kolthoff and Lingane (1952), "When an electrolyte solution is electrolysed in a cell consisting of a dropping mercury electrode and a second non-polarizable electrode, it is possible to determine from the resulting current-voltage curve both the nature and the concentration of the reducible or oxidizable substance, or substances, present."

Because of the unique properties which give it inherent advantages over solid electrodes, the dropping mercury electrode is employed almost exclusively in conventional polarographic work, as for example, in the detection and quantitative measurement of mixtures of metallic ions in dilute aqueous solutions. These properties are: (a) a constantly renewed electrode surface which avoids the build-up of coatings of reduced substances, and (b) the relative motion of the electrode and the electrolyte is accomplished simply. In conventional polarographic work in which molecular oxygen is not the substance to be analyzed, atmospheric contamination of the electrolyte of the polarographic cell with dissolved oxygen (which is readily reduced) is guarded against by purging the cell with an inert gas such as nitrogen before, but not during, an analysis.

The dropping mercury electrode has been used by many investigators, with varying degrees of success, to monitor the dissolved oxygen saturation in natural and waste waters (Manning, 1950; Moore, Morris, and Okun, 1948; Seaman and Allen, 1950; and Wood, 1953) and in laboratory studies of respiring biological systems (Knowles, Edwards, and Briggs, 1963; Petering and Daniels, 1938; and Edwards and Learner, 1960). For monitoring dissolved oxygen in natural waters, the most advantageous modification of the dropping mercury electrode appears to be the wide bore type developed by Briggs, Dyke, and Knowles (1958).

A development in polarographic technique introduced by Heyrovsky and Berezicky in 1929 (Heyrovsky, 1960) directly anticipated modern electrochemical oxygen transducers. The introduction of polarographic titrations, later called amperometric titrations by Kolthoff (1939), uses the limiting current to indicate the end point. As a result of this development, the indicating electrode could be a rotating or vibrating electrode of solid metal. The dropping mercury electrode, as well as the vibrating and rotating electrodes which will be discussed subsequently, has shortcomings. Easily reduced materials such as the ions of iron, copper, zinc, and nickel and polar organic substances such as surface active agents will affect the cell current and in instances "poison" the electrode surfaces. Deposits of hard water scale, turbulence, and temperature changes may be troublesome. Also, mercury is poisonous to biological systems. This limits the usefulness of this type of electrode in laboratory oxygen metabolic studies.

#### c. Present Design of Electro-Chemical Oxygen Transducers

It remained for other investigators such as Davies and Brink (1942) and Clark, Weld, and Taylor (1953) to introduce "covered" solid electrodes for the measurement of dissolved oxygen in biological systems. "Covered" electrodes have their electrode surfaces protected from the electrolytes and oxidizable or reducible substances by a hydrophobic, gas permeable, ion impermeable membrane which encloses an interstice containing a supporting electrolyte. The shape and relative geometry of the cathode and anode were modified; the general design now has a disk-shaped cathode separated from but surrounded by an annular anode. The cathode is kept at approximately 0.8 volt negative with respect to the anode. The membrane permits the passage of oxygen and other gases from the outer environment into the interstice containing the supporting electrolyte, but prevents the passage of ions and large molecules. The molecular oxygen is reduced after it diffuses through the membrane and interstitial electrolyte, while corresponding oxidation reaction is taking place at the anode. This arrangement permits the device to operate under most conditions, as a selective oxygen transducer. It produces an electrical current directly proportional to the oxygen concentration in the fluid layer immediately adjacent to the outer membrane surface, other parameters being constant. The device is diffusion controlled so that the flow velocity of the medium past the membrane, below a minimum velocity, will affect the cell current. In addition, there is a pronounced temperature coefficient of the cell current, which represents a combination of the effects of temperature on the diffusion and the solubility of oxygen and also the temperature kinetics of the chemical reactions involved. Thus, minimum flow velocity conditions must be met, and the temperature coefficient of response must be corrected either by calibration and simultaneous temperature readings or through internal compensation with thermistors.

Many electrode couples have been developed into commercially available instruments. One of these, the Beckman Model 777 oxygen analyzer uses a gold cathode which is maintained at 0.8 volt negative with respect to a silver-silver chloride reference electrode. The electrolyte used is a gel containing the chloride ion. The electro-chemical reaction which takes place during the reduction of oxygen results in the disappearance of chloride ions at the anode and the appearance of hydroxyl ions at the cathode. The decrease in concentration of the chloride ions causes gradual decrease in cell current, consequently frequent recharging of the cell electrolyte is necessary. To a limited degree CO<sub>2</sub> and gases such as the halogens will give erroneous readings. Hydrogen sulfide and mercaptans irreversibly poison the electrode.

A platinum cathode maintained at 0.8 volt negative with respect to a silver-silver oxide reference electrode, enveloped in a polyethylene or teflon membrane containing a KOH solution, constitute the electrode couple proposed by Carritt and Kanwisher (1959). This electrode is insensitive to chlorine, hydrogen sulfide, and pH changes between 2 and 12 in the external environment. The cathode reaction renews in exact measure the electrolyte used at the anode, avoiding concentration changes in the internal environment.

#### d. Optional Respirometer Designs

With an electro-chemical oxygen transducer, it is possible to design several types of respirometers. For example, the transducer can be placed in a "closed" system containing respiring biological materials and an oxygen depletion curve can then be obtained. The slope of the curve indicates the rate of oxygen uptake by the system (Mancy and Westgarth, 1962; Kanwisher 1959). Alternatively, a "closed loop or circulating pipe" arrangement with a continuous measurement of oxygen saturation would permit study of effects of flow velocity upon respiration rates of aquatic biological specimens (Jaag and Ambuhl, 1963).

The "closed loop" system of respirometry can be modified to a single pass arrangement such as used by Kanwisher (1959) and Bielawski (1961). If the rate of flow through the chamber and the absolute concentration of dissolved oxygen in the influent and effluent stream are known, an oxygen balance can be established which permits the expression of the oxygen utilization by organisms or materials within the chamber. One may also add a potentiometric recorder to the system.

### 5. Manometric Methods

Manometric methods were developed by Barcroft, Haldane, Warburg, and others during the period 1900-1930 in connection with investigations made in the fields of microbiology and pathology (Ludwig, Oswald, and Gotaas, 1951); the application to other fields was furthered by the work of Dixon (1943). These methods rely on the principle that if an organism is placed in an enclosed space and the carbon dioxide produced is absorbed by an alkaline solution, the gas pressure within the space will decrease as oxygen is used. If the space is connected with a manometer, the recorded decrease in pressure gives a measure of the oxygen uptake of the organism. Three general methods of manometric respirometry have evolved. These involve measurement of: (a) change in pressure at constant volume, as in the commonly called "Warburg" instrument; (b) changes in volume at con-

stant pressure, as in the Haldane type; and (c) changes in both pressure and volume, as in the differential type respirometers of Barcroft and of Fenn.

Two types of constant-volume differential manometers have been developed which combine the advantages of the Warburg constant volume type and the Barcroft differential type. One of these was described by Dickens and Greville (1933) and the other type by Summerson (1939). Gilson (1963) described a constant pressure respirometer which is available commercially. The open arms of all the manometers are connected to a common compensation vessel through a manifold. This vessel eliminates corrections for barometric changes and minimizes temperature errors. The apparatus provides stability comparable to that of the Barcroft respirometer and considerably better than that of the Warburg; the vessels require no calibration. Burk and Hobby (1954) suggested the use of hydraulic-leverage principles combined with unusual moving-boundary indices to increase the sensitivity of the conventional Warburg 10-to-100-fold. Automatic recording respirometers, which are attachable to standard Warburg manometers and will read, calculate, and record gas exchange in  $\mu\text{l}$  gas without the usual continual attention by the operator, have become commercially available recently. According to Umbreit, Burris, and Stauffer (1964) the "Manomatic" by Otto Koelle of Germany is less expensive than the "Mechrolab" developed by R. E. Steele of Mechrolab, but requires more attention and is subject to greater fluctuations. Although the cost may be well over \$1000 a manometer, with these, calculations are almost eliminated, experiments are speeded up several fold, greater precision is possible, and the continuous recording gives one a more precise picture of what is happening.

For measuring less exchange than can be measured accurately with the Warburg instrument ( $10 \mu\text{l}$  per hour), micro- and ultramicro-respirometers have been developed. Those employing the Cartesian-diver principle, described by Holter and Linderstrom-Lang in 1943 (Glick, 1961), Anfinsen and Claff (1947), and Holter and Linderstrom-Lang (1951) have a sensitivity of about  $2 \times 10^{-3} \mu\text{l}$  per hour. With this method, a gas bubble is enclosed within a small glass vessel and this is immersed in fluid within a larger vessel. The buoyancy of the small vessel (diver) will vary according to the pressure imposed on the fluid in the larger vessel; the diver will sink or rise as this pressure rises or falls. The diver can thus serve as a constant volume manometer if from time to time it is brought to a definite level (the zero line) and the pressure required to do so is noted. The divers have glass tails that maintain them in a vertical position. In the micro-manometers, the organism is placed in a glass capillary; a droplet of alkaline solution absorbs the carbon dioxide. An oil droplet measures the decrease of the volume of the air space and also serves to seal one end of the air space. Gregg (1947) described a microrespirometer having a sensitivity comparable to the Cartesian diver, but simpler in operation. This was a modification of the Warburg constant volume respirometer and the calculations required for converting the pressure readings into volume were the same as for the Warburg. A droplet of Brodie's solution in a capillary served as the index droplet, and a microscope was used to return the index droplet to the zero point. Prop (1954) described a microrespirometer capable of simultaneously measuring  $\text{O}_2$  consumption and  $\text{CO}_2$  production. This was based on the combination and improvement of Gregg's instrument and the measurement of  $\text{CO}_2$  production by the change in electrical conductivity of a  $\text{CO}_2$ -absorbing  $\text{Ba}(\text{OH})_2$  solution. An Intercon differential microrespirometer kit for assembling is now available; this is a modification of the Cruickshank (1954) respirometer.

Manometric methods are simpler when the living material is in direct contact with air. Many aquatic invertebrates depend on dissolved oxygen; the rate of oxygen uptake by the organism must not be greater than can be replaced by the diffusion of oxygen from the atmosphere into the fluid. Otherwise the rate of respiration will be dependent upon the rate at which oxygen diffuses into the fluid and not on the potential respiration rate of the organism. To obtain a fluid phase saturated with the gas phase, some method of adequately shaking the fluids in the respirometer is necessary. The CO<sub>2</sub> liberated in the respiration process must be completely and instantaneously absorbed, or the CO<sub>2</sub> pressure in the gas phase will not be zero and the readings on the manometer will not represent the true oxygen uptake. Since the alkali is confined to a small section of the respirometer, the rate of shaking has little effect on increasing the surface. Folded filter papers are used in the wells to increase the surface of the alkali. In the respect of CO<sub>2</sub> absorption especially when making readings at 5-10 minute intervals, Punt (1950-52) cautions that results with the Warburg or Barcroft method will be quite different depending upon whether the measurement is performed during an interval between the CO<sub>2</sub> peaks or during a CO<sub>2</sub> outburst. He believes this may account for considerable individual variation and variation from time to time of one individual. In using the Warburg with adult insects, Punt, Parser, and Kuchlein (1957) found that sometimes the curves of oxygen consumption rise above the zero line which would indicate that there is in this interval an increase in pressure in comparison with that of the preceding reading. This can only mean that at these moments CO<sub>2</sub> bursts occur and the CO<sub>2</sub> absorption is not yet completed. But as the CO<sub>2</sub> is readily absorbed, the pressure is considerably decreased in the following 5-minute period, accentuating the apparent O<sub>2</sub> uptake in this period.

## 6. Volumetric Methods

Using respirometers, this method gives direct measurement of gas volume without the necessity of determining the volume of the apparatus. As with the manometric methods, the carbon dioxide is absorbed. Oxygen is introduced to keep the volume constant and the pressure is balanced against a compensating vessel. The respiratory vessels can be of any form, but if maximal accuracy is desired, the gas volume should be kept small (Scholander, 1942).

The volumetric respirometers described by Winterstein (1912) and modified and improved by Dixon (1943), Scholander (1942), and Scholander and Edwards (1942) have not been as popular as the Warburg and Barcroft respirometers even though they have inherent advantages (Umbreit, et al, 1964) and greater sensitivity (Glick, 1961). A Scholander modification is now commercially available. The Scholander process also can be used to determine dissolved oxygen in water. The method consists of sparging the water with nitrogen and collecting the gas, after which it can be subjected to gas analysis in a modified Scholander micro-analytical apparatus.

Sandeen, Stephens, and Brown (1955) used a respirometer consisting of a plastic bag of oxygen attached to a soxhlet flask by a capillary connection and a continuously recording apparatus to measure the oxygen consumption of marine snails. As the oxygen was consumed, the buoyancy of the bag decreased; the increase in the specific gravity of the chamber was measured by means of a lever system attached to the ink-writing pen of the recorder. The submerged diver increased in weight by 1 gm for each milliliter of oxygen utilized, and the extent of excursion of the pen per hour could be converted into amounts of oxygen consumed.

TABLE 1. Investigations on the oxygen consumption of immature aquatic insects.

| INVESTIGATOR(S)<br>CHEMICAL METHOD (WINKLER) | IMMATURE AQUATIC INSECT                                                                                                                                                                                                                                                                                                                               | TYPE OF INVESTIGATION                                                                                                                                                                                                               |
|----------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Collardeau, 1962                             | Trichoptera<br>: <u>Polycentropus flavonaculatus</u> Pict.<br>: <u>Plectrocnemia conspersa</u> (Curt.)<br>: <u>Limnophilus rhombicus</u> (L.)<br>: <u>Chironomus</u> sp.                                                                                                                                                                              | Effect of temperature;<br>running H <sub>2</sub> O and stagnant H <sub>2</sub> O<br>species.<br><br>Function of hemoglobin; effect<br>of normal and low O <sub>2</sub> concen-<br>trations.                                         |
| Ewer, 1942                                   | Diptera                                                                                                                                                                                                                                                                                                                                               | Flowing and still H <sub>2</sub> O species;<br>effect of O <sub>2</sub> concentration;<br>effect of size.                                                                                                                           |
| Fox, Wingfield, and Sim-<br>monds, 1937      | Ephemeroptera<br>: <u>Cloeon diaterum</u> L.<br>: <u>Ephemera vulgata</u> L.<br>: <u>Leptophlebia marginata</u> L.<br>: <u>Leptophlebia vespertina</u> L.<br>: <u>Baetis scambus</u> Eaton<br>: <u>Baetis</u> sp.                                                                                                                                     |                                                                                                                                                                                                                                     |
| Hiestand, 1931                               | Odonata<br>Trichoptera<br>: <u>Nasiaeschna penthacantha</u><br>: <u>Limnephilus rhombicus</u>                                                                                                                                                                                                                                                         | Effect of lowering O <sub>2</sub> concen-<br>trations; effect of pH; effect<br>of high CO <sub>2</sub> content.<br><br>Normal and de-gilled;<br>effect of O <sub>2</sub> concentration;<br>effect of CO <sub>2</sub> concentration. |
| Morgan and O'Neil, 1931                      | Trichoptera<br>: <u>Macronema zebratum</u> Hagen                                                                                                                                                                                                                                                                                                      | Importance of caudal lamellae.                                                                                                                                                                                                      |
| Munchberg, 1962                              | Odonata<br>: <u>Agriion puella</u> (L.)<br>: <u>Enallagma cyathigerum</u> (Charp.)<br>: <u>Erythronma naias</u> (Hansemann)<br>: <u>Ischnura elegans</u> (Vanderl.)<br>: <u>Lestes sponsa</u> (Hansemann)<br>: <u>Lestes virens</u> (Charp.)<br>: <u>Pyrrhosoma nymphula</u> (Sulzer)<br>: <u>Aeschna cyanea</u> Mull.<br>: <u>Aeschna grandis</u> L. |                                                                                                                                                                                                                                     |
| Walshe, 1948                                 | Diptera<br>: <u>Chironomus longistylus</u><br>: <u>Anatopymia nebulosa</u><br>: <u>Anatopymia varia</u><br>: <u>Tanytarsus brunripes</u>                                                                                                                                                                                                              | Flowing and still H <sub>2</sub> O forms;<br>decreased O <sub>2</sub> content; re-<br>sistance to anaerobic condi-<br>tions; thermal resistance.                                                                                    |

| INVESTIGATOR(S)                                          | IMMATURE AQUATIC INSECT                                                                                                                                                     | TYPE OF INVESTIGATION                                                                                              |
|----------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------|
| Wingfield, 1939                                          | Ephemeroptera<br>: <u>Baetis</u> sp.<br>: <u>Cloeon dipterum</u> L.<br>: <u>Ephemera vulgata</u> (L.)                                                                       | Normal and de-gilled at various $O_2$ concentrations; effect of habitat of species.                                |
| Woynabovich, 1961                                        | Ephemeroptera<br>: <u>Ephemera danica</u>                                                                                                                                   | Effect of temperature and size.                                                                                    |
| ELECTRO-CHEMICAL METHOD                                  |                                                                                                                                                                             |                                                                                                                    |
| Jaag, 1955                                               | Ephemeroptera<br>Trichoptera<br>: <u>Ecdyonurus</u><br>: <u>Ephemerella</u><br>: <u>Hydropsyche</u>                                                                         | In closed system; survival at different $O_2$ concentrations and water flow rates.                                 |
| Mancy, Okun & Reilley, 1962<br>Mancy and Westgarth, 1962 | Odonata<br>: <u>Tetragoneuria cynosura</u>                                                                                                                                  | In a closed system.                                                                                                |
| MANOMETRIC METHOD (WARBURG)                              |                                                                                                                                                                             |                                                                                                                    |
| Edwards, 1958                                            | Diptera<br>: <u>Chironomus riparius</u>                                                                                                                                     | Effect of temperature and body size; summer and winter forms.                                                      |
| Hinton, 1964                                             | Diptera<br>: <u>Simulium ornatum</u> pupae                                                                                                                                  | Differences in age of pupae.                                                                                       |
| Norris, Arnold, and Edwards<br>1964                      | Trichoptera<br>: <u>Cheumatopsyche comis</u><br>: <u>Chimarra betteni</u><br>: <u>Notiomyia ornata</u>                                                                      | Effect of temperature, increased $O_2$ concentration ( $O_2$ poisoning), and weight; cool and warm $H_2O$ species. |
| MANOMETRIC METHOD (BARCROFT)                             |                                                                                                                                                                             |                                                                                                                    |
| Fox and Simmonds, 1933                                   | Ephemeroptera<br>Trichoptera<br>: <u>Baetis rhodani</u><br>: <u>Chloeon dipterum</u><br>: <u>Hydropsyche</u> sp.<br>: <u>Limnephilus vittatus</u><br>(= <u>Molanna</u> sp.) | Swift and slow stream (or pond) species.                                                                           |

| INVESTIGATOR(S)                    | IMMATURE AQUATIC INSECT                                                                                                                                                                                            | TYPE OF INVESTIGATION                                                             |
|------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|
| Fox, Simmonds, and Washbourn, 1935 | Ephemeroptera<br>: <u>Caenis</u> sp.<br>: <u>Chloen</u> dipterum<br>: <u>Ecdyonuris</u> venosus<br>: <u>Ephemerella</u> <u>ignita</u><br>: <u>Ephemerella</u> <u>danica</u><br>: <u>Ephemerella</u> <u>vulgata</u> | Swift H <sub>2</sub> O and pond species; resistance to O <sub>2</sub> deficiency. |
| VOLUMETRIC METHOD<br>Edwards, 1946 | Odonata<br>: genus not given                                                                                                                                                                                       | Effect of temperature.                                                            |

## E. BIBLIOGRAPHY

- Allee, W. C. and R. Oesting, 1934. A critical examination of Winkler's method for determining dissolved oxygen in respiration studies with aquatic animals. *Physiol. Zool.* 7:509-541.
- Anfinsen, C. B. and C. L. Claff, 1947. An extension of the Cartesian diver micro respirometer technique. *J. Biol. Chem.* 167:27-33.
- Bielawski, J., 1961. The use of a solid platinum electrode for continuous recording of the rate of respiration. *Comp. Biochem. Physiol.* 3:261-266.
- Briggs, R., G. V. Dyke, and G. Knowles, 1958. Use of the wide bore dropping-mercury electrode for long-period recording of concentration of dissolved oxygen. *The Analyst* 83:304-311.
- Brinkman, R. and A. Van Schreven, 1942. A simple colorimetric micro-method for determining the oxygen content of watery solutions. *J. Exptl. Biol.* 19:1-4.
- Burk, D. and G. Hobby, 1954. Hydraulic-leverage principles for magnification of sensitivity of gas change in free and fixed-volume manometry. *Science* 120:640-648.
- Carritt, D. E. and J. W. Kanwisher, 1959. An electrode system for measuring dissolved oxygen. *Anal. Chem.* 31:5-9.
- Clark, L. C. Jr., R. C. Weld, and Z. Taylor, 1953. Continuous recording of blood oxygen tension. *J. Appl. Physiol.* 6:189-193.
- Collardeau, C., 1962. Influence de la temperature sur la consommation d'oxygene de quelques larves de Trichopteres. *Hydrobiol. Acta Hydrobiol. Hydrogr. Protistol.* 18:252-264.
- Cruickshank, C. N. D., 1954. Continuous observation of the respiration of skin in vitro. *Exp. Cell Research* 7:374-380.
- Davies, P. W. and F. Brink, Jr., 1942. Micro-electrodes for measuring local oxygen tension in animal tissues. *Rev. Sci. Instr.* 13:524-533.
- Dickens, F. and G. D. Greville, 1933. A constant volume differential manometer. *Biochem. J.* 27:213-219.
- Dixon, M., 1943. *Manometric methods as applied to the measurement of cell respiration and other processes.* 2nd ed. Univ. Press, Cambridge, England. 122 pp.
- Edwards, G. A., 1946. The influence of temperature upon the oxygen consumption of several arthropods. *J. Cell. Comp. Physiol.* 27:53-64.
- Edwards, R. W., 1958. The relation of oxygen consumption to body size and to temperature in the larvae of Chironomus riparius Meigen. *J. Exptl. Biol.* 35:383-395.
- Edwards, R. W. and M. A. Learner, 1960. Some factors affecting the oxygen consumption of Asellus. *J. Exptl. Biol.* 37:706-718.
- Ewer, R. F., 1942. On the function of haemoglobin in Chironomus. *J. Exptl. Biol.* 18:197-205.

- Fox, H. M. and B. G. Simmonds, 1933. Metabolic rates of aquatic arthropods from different habitats. *J. Exptl. Biol.* 10:67-74.
- Fox, H. M., B. G. Simmonds, and R. Washbourn, 1935. Metabolic rates of ephem-  
erid nymphs from swiftly flowing and from still waters. *J. Exptl. Biol.* 12:  
179-184.
- Fox, H. M. and C. A. Wingfield, 1938. A portable apparatus for the determina-  
tion of oxygen dissolved in a small volume of water. *J. Exptl. Biol.* 15:  
437-445.
- Fox, H. M., C. A. Wingfield, and B. G. Simmonds, 1937. The oxygen consumption  
of ephemerid nymphs from flowing and from still waters in relation to the  
concentration of oxygen in the water. *J. Exptl. Biol.* 14:210-218.
- Gaufin, A. R. and C. M. Tarzwell, 1952. Aquatic invertebrates as indicators of  
stream pollution. *Public Health Repts. (U.S.)* 67:57-64.
- Gaufin, A. R. and C. M. Tarzwell, 1956. Aquatic macro-invertebrate communities  
as indicators of organic pollution in Lytle Creek. *Sewage and Indust.*  
*Wastes* 28:906-924.
- Gilson, W. E., 1963. Differential respirometer of simplified and improved  
design. *Science* 14:531-532.
- Glick, D., 1961. Quantitative chemical techniques of histo- and cytochemistry.  
Vol. 1. Interscience Publ., New York and London. 470 pp.
- Gregg, J. R., 1947. A microrespirometer. *Rev. Sci. Instr.* 18:514-516.
- Heyrovsky, J., 1941. Polarography. Wein, Springer. 514 pp.
- Heyrovsky, J., 1960. Trends in polarography. *Science* 132:123-130.
- Hiestand, W. A., 1931. The influence of varying tensions of oxygen upon the  
respiratory metabolism of certain aquatic insects and the crayfish.  
*Physiol. Zool.* 4:246-270.
- Hinton, H. E., 1964. The respiratory efficiency of the spiracular gill of  
Simulium. *J. Insect Physiol.* 10:73-80.
- Holter, H. and K. Linderstrom-Lang, 1951. Micromethods and their application  
in the study of enzyme distribution in tissues and cells. *Physiol. Rev.*  
31:432-448.
- Hynes, H. B. N., 1960. The biology of polluted waters. Liverpool Univ. Press.  
165 pp.
- Jaag, O., 1955. Die verunreinigung von fliessgewassern, dargestellt am bei-  
spiel des Rheinstroms. *Ber. Abwassertechin. Verein. Oldenbourg, Munich*  
(Germany) No. 6 R:41-61.
- Jaag, O. and H. Ambuhl, 1963. The effect of the current on the composition of  
biocoenoses in flowing water streams. *Inter. J. Air Water Poll.* 7:317-330.
- Kanwisher, J., 1959. Polarographic oxygen electrode. *Limn. and Oceanog.* 4:  
210-217.

- Keys, A. B., 1930. The measurement of the respiratory exchange of aquatic animals. *Biol. Bull.* 59:187-198.
- Knowles, G., R. W. Edwards, and R. Briggs, 1962. Polarographic measurement of the rate of respiration of natural sediments. *Limn. and Oceanog.* 7:481-484.
- Kolthoff, I. M. and Y. D. Pan, 1939. Amperometric (polarometric) titrations. I. The amperometric titration of lead with dichromate or chromate. *J. Am. Chem. Soc.* 61:3402-3409.
- Kolthoff, I. M. and J. J. Lingane, 1952. *Polarography*. 2nd ed. Interscience Publ., New York. 553 pp.
- Kolkwitz, R., 1950. Oekologie der saprobien. *Schriftenreihe des Vereins für Wasser-, Boden- und Lufthygiene*, Nr. 4. Piscator-Verlag Stuttgart. 64 pp.
- Kolkwitz, R. and M. Marsson, 1908. Oekologie der pflanzlichen saprobien. *Ber. d. Deut. Bot. Gesell.* 26a:505-519.
- Koltwitz, R. and M. Marsson, 1909. Oekologie der tierischen saprobien. *Int. Rev. d. ges. Hydrog. u. Hydrog.* 2:126-152.
- Ledig, P. G. and R. S. Lyman, 1927. An adaptation of the thermal conductivity method to the analysis of respiratory gases. *J. Clin. Invest.* 4:495-505.
- Liebmann, H., 1951. *Handbuch der frischwasser- und abwasserbiologie*. Vol. 1. Oldenbourg, München (Germany). 539 pp.
- Liebmann, H., 1960. *Handbuch der frischwasser- und abwasserbiologie*. Vol. 2. R. Oldenbourg, München (Germany). 1149 pp.
- Levine, H. S., W. V. Warren, E. C. Tsivoglou, and W. W. Walker, 1956. Continuous measurement of dissolved oxygen in water. *Anal. Chem.* 28:343-349.
- Ludwig, H. F., W. J. Oswald, and H. B. Gotaas, 1951. Manometric technique for measurement of BOD. *Sanit. Eng. Lab., Univ. Calif., Inst. Eng. Research, Berkeley*. 31 pp.
- Mancy, K. H., D. A. Okun, and N. Reilley, 1962. A galvanic cell oxygen analyzer. *J. Electroanal. Chem.* 4:65-92.
- Mancy, K. H. and W. C. Westgarth, 1962. A galvanic cell oxygen analyzer. *J. Water Poll. Control Feder.* 34:1037-1051.
- Manning, W., 1940. A method for obtaining continuous records of dissolved oxygen in lake waters. *Ecology* 21:509-512.
- Moore, E. W., J. C. Morris, and D. A. Okun, 1948. A polarographic determination of dissolved oxygen in water and sewage. *Sewage and Indust. Wastes* 20:1041-1053.
- Morgan, A. H. and H. D. O'Neill, 1931. The function of the tracheal gills in larvae of the caddis fly, Macronema zebratum Hagen. *Physiol. Zool.* 4: 361-379.

- Munchberg, P., 1962. Vergleichende untersuchungen uber die atmungsintensitat der zygopteren-larven, zugleich ein experimenteller beitrag zur funktionellen bedeutung ihrer analblatter (Odonata). *Beit. z. Entomol.* 12:243-270.
- Norris, W. E., C. R. Arnold, and S. W. Edwards, 1964. Oxygen consumption by caddisfly larvae. *Tex. J. Sci.* 16:72-79.
- Olson, T. A., 1957. Biological indicators in stream ecology. *Eng. Bull., Proc. 11th Indust. Waste Conf.*, 15-17 May 1957 XLI:601-610.
- Petering, H. G. and F. Daniels, 1938. The determination of dissolved oxygen by means of the dropping mercury electrode, with applications in biology. *J. Am. Chem. Soc.* 60:2796-2802.
- Prop, F. J. A., 1954. A microrespirometer for simultaneous measurement of O<sub>2</sub>-consumption and CO<sub>2</sub>-production and its use in determining the respiratory quotient of tissue cultures. *Exptl. Cell Research* 7:303-317.
- Punt, A., 1950. The respiration of insects. *Physiol. Comp. et Oecol.* 2:59-74.
- Punt, A., W. J. Parser, and J. Kuchlein, 1957. Oxygen uptake in insects with cyclic CO<sub>2</sub> release. *Biol. Bull.* 112:108-119.
- Sandeen, M. I., G. C. Stephens, and F. A. Brown, Jr., 1955. Persistent daily and tidal rhythms of oxygen consumption in two species of marine snails. *Physiol. Zool.* 38:350-356.
- Scholander, P. F., 1942. Volumetric microrespirometers. *Rev. Sci. Instr.* 13:32-33.
- Scholander, P. F. and G. A. Edwards, 1942. Volumetric microrespirometer for aquatic organisms. *Rev. Sci. Instr.* 13:292-295.
- Seaman, W. and W. Allen, 1950. Polarographic determination of dissolved oxygen in water containing industrial effluent. *Sewage and Indust. Wastes* 22:912-921.
- Standard Methods, 1960. Standard methods for the examination of water and wastewater including bottom sediments and sludges. 11th ed. Am. Public Health Assoc. Inc., New York. 626 pp.
- Summerson, W., 1939. A combination simple manometer and constant volume differential manometer for studies in metabolism. *J. Biol. Chem.* 131:579-595.
- Umbreit, W. W., R. H. Burris, and J. F. Stauffer, 1964. *Manometric techniques*. 4th ed. Burgess Publ. Co., Minneapolis. 305 pp.
- Usinger, R. L. (Editor), 1956. *Aquatic insects of California*. Univ. Calif. Press, Berkeley. 508 pp.
- Van Dam, L., 1935. A method for determining the amount of oxygen dissolved in 1 c.c. of water. *J. Exptl. Biol.* 12:80-85.
- Walshe, B. M., 1948. The oxygen requirements and thermal resistance of chironomid larvae from flowing and from still waters. *J. Exptl. Biol.* 25:35-44.

- Weaver, E. R., P. E. Palmer, H. Frantz, P. C. Ledig, and S. F. Pickering, 1920. Automatic methods of gas analysis depending upon thermal conductivity. J. Indust. Eng. Chem. 12:359-365.
- Wingfield, C. A., 1939. The function of the gills of mayfly nymphs from different habitats. J. Exptl. Biol. 16:363-373.
- Winterstein, H., 1912. Ein apparat zur mikroblutgasanalyse und mikrorespirometric. Biochem. Zeits. 46:440-449.
- Wolvekamp, H. P. and T. A. Waterman, 1960. Respiration. In The physiology of crustacea, Vol. 1, edited by T. H. Waterman. Academic Press, New York. 670 pp.
- Wood, K. G., 1953. Polarograms of oxygen in lake water. Science 117:560-561.
- Woynarovich, E., 1961. Sauerstoffverbrauch einiger wassertiere bei verschiedener temperatur. Verh. Intern. Verein. Limn. 14:1014-1018.

## II. ELECTRO-CHEMICAL METHOD AS USED IN THIS INVESTIGATION

### A. OBJECTIVES

The objectives of this phase of the investigation were: (1) supplementary studies on the oxygen consumption of aquatic immature insects using the electro-chemical method and a flow-through respirometry chamber since this method more nearly simulates natural conditions in a stream, a continuous record of the respiration is possible, and the factor of water movement can be studied; (2) evaluation of the dissolved oxygen, temperature, and flow fluctuations to which benthonic invertebrates are exposed in the field; and (3) fabrication of galvanic cells for special uses.

### B. PROCEDURE

#### 1. Flow-Through Respirometer

Reproduced from  
best available copy.

The flow-through, or single pass type of respirometer previously discussed (Section I), was chosen for this study because of its advantages over "closed" systems. Initially, in our prototype flow-through respirometer a Jarrell-Ash oxygen analyzer was used as a sensor to measure the respiration rates of immature aquatic insects from the orders Odonata, Trichoptera, and Diptera. Three to eight specimens were used per test. Distilled, low-conductivity water was used as the aqueous medium and no "screen substratum" to which the insects might cling was provided in the respiratory chamber.

As a result of these preliminary experiments the apparatus was redesigned to measure the oxygen consumption of single specimens as shown in Plates 1 and 2. In redesigning the prototype, the following changes were made: (a) all the connecting tubing was reduced in lumen and length to decrease the dead volume and hence the response time of the system; (b) the respiratory chamber was replaced by two smaller optional chambers with different cross sectional areas (C.S.A.) which permitted selection of a variety of cross sectional flow velocity rates (see Fig. 4); (c) in order to provide more normal conditions, a substratum of polyurea screen was added to the respiration chamber (Fig. 4); (d) the battery operated Jarrell-Ash oxygen analyzer was replaced with a line-operated Beckman analyzer. The Beckman instrument, although it required more frequent changing of the sensor electrolyte than the Jarrell-Ash, was found to be adequate after the substitution of a wire wound potentiometer for a carbon composition span control, and this freed the battery-operated Jarrell-Ash instrument for use in field studies (Fig. 7 and 8; Plate 5); (e) the distilled unbuffered water used previously was replaced by "synthetic" natural water\*; (f) the reservoir supply manifold ("C" in Fig. 1) was fitted with an additional port ("M" in Fig. 1) to facilitate the measurement of reservoir pH and the collection of "Micro-winkler" samples for the calibration of the recorder span adjustment, and (g) a mineral oil sealed sulfite solution trap ("H" in Fig. 1) was added to the system to prevent the pick up of atmospheric oxygen by the sulfite solution in the reservoir. The oxygen-free sulfite solution is used to set the instrument zero of the oxygen analyzer and the recorder.

---

\* This was prepared as follows: (a) approximately 20 gm of solid carbon dioxide was added to 20 liters of distilled, deionized water in a Polyethylene carboy; (b) after 5 minutes, 50 mg per liter of calcium carbonate was added to the carboy, and the solution was aerated for at least 18 hours with compressed atmospheric air.

Utilizing the redesigned equipment, the procedure for experiments was as follows: (a) single immature aquatic insects of the orders Odonata (Anisoptera) and Plecoptera were used as subjects; (b) two series of respiration measurements were made on different days. Each series consisted of three measurements in each of the two aforementioned chambers of differing cross sectional area. The three measurements of a series were made at flow rates of approximately 6, 3, and 1.2 ml per minute; (c) each measurement of oxygen consumption rate was made during a forty minute period, and the oxygen consumption was determined by measuring the area under the respiration tracing with a polar planimeter; (d) before each series of measurements, the experimental insects were kept for at least a week in a limestone-buffered three liter holding aquaria in the same temperature controlled room as the respirometer.

## 2. Portable Field Equipment for Monitoring Dissolved Oxygen Saturation and Temperature

To satisfy the overall objectives of this study, it was considered desirable to evaluate the dissolved oxygen saturation, temperature, and flow fluctuations to which benthonic invertebrates are exposed. The importance of direct measurements in the field is generally recognized. Jaag and Ambuhl (1963) point out that the temperature, current, and chemistry of the water exert great influence on the composition of living communities.

The portable unit we have assembled for determination of temperature and dissolved oxygen is shown in Figure 7 and Plate 5. This unit is designed to give a continuous record of these two parameters of water quality. The electrode couple used is that proposed by Carritt and Kanwisher in 1959 (see Section I). In adapting the electronic thermistor thermometer and the Jarrell-Ash analyzer to field use, it was necessary to make these instruments compatible with the portable Esterline Angus recorder because the latter essentially is a microammeter and the output of the Jarrell-Ash and the thermistor thermometer are meant for potentiometric recorders. In addition, the clock-work chart drive mechanism of the Esterline-Angus instrument was fitted with a cam-actuated, double-pole double-throw micro-switch so that two signals could be recorded serially. One signal for 88 per cent of 10 minutes could record temperature or oxygen saturation, and the other signal for 12 per cent of 10 minutes could also record temperature or oxygen saturation.

This field equipment was subjected to two laboratory "shake down" trials and one field trial. In the first trial, a 10 gallon laboratory sink in a thermostatically controlled room was monitored for 17 hours without making adjustments to the calibrations. The second laboratory trial was conducted in essentially the same manner except the period was 19 hours, the sink of 3 gallon capacity, and the temperature of the water was previously equilibrated with room temperature.

## C. RESULTS

### 1. Studies of Oxygen Uptake of Odonata and Plecoptera Naiads Utilizing the Flow-Through Respirometer

Plates 3 and 4 show typical respiration tracings obtained during the study. "R" represents the calibration interval and "S" the interval of the zero check; the distance between "R" and "S" is the span. "R", therefore, represents the dissolved oxygen concentration of the water as it enters the test chamber. "U", "V", "X", and "Y" represent dissolved oxygen saturation in the effluent from

the test chamber. The 720 mg Plecoptera (stonefly) naiad whose tracings appear in Plates 3 and 4 had been "overwintered" two or more months longer in the laboratory than the Odonata (Anisoptera) naiad, or for a total of four months. A freshly collected (in the laboratory three days) Plecoptera naiad of the same species weighing 920 mg showed a much more irregular tracing and had an oxygen consumption about double that of the overwintered specimen. The tracing from the freshly collected specimen, therefore, more nearly resembled that obtained from the dragonfly (Odonata).

In Figures 6a, b, c, and d the ordinates of the four graphs are metabolic rates. The statistical mean and standard deviations of the temperatures and pH's during the measurements are indicated. Abbreviations A. vert. for Aeshna verticalis and P. pictetii for Pteronarcys pictetii are used in the figures. Figures 6a and b appear to suggest that a flowing environment may increase the respiration rate of stonefly naiads, but no such trend is seen in the corresponding graph from dragonfly (Anisoptera) naiads. In Figure 6c, which relates oxygen uptake and body weight, a trend is apparent towards an increasing metabolic rate with a decrease in size in the case of the dragonfly (Anisoptera) naiads, if the overwintered subjects are ignored. The effect that the length of holding time in the laboratory had on the metabolic rates of dragonflies and stoneflies is shown in Figure 6d. The oxygen consumption rate definitely decreased with the length of time in the insectary. The lowest rates in December were approximately in the range of the highest obtained in April. The factors bringing this about have not yet been explored.

## 2. Studies with Portable Field Equipment for Continuous Measurement of Dissolved Oxygen Saturation and Temperature

Utilizing the field equipment shown on Plate 5, the oxygen saturation and temperature fluctuations were recorded in a lagoon at Elk River on May 25-26. These records are shown in Figure 8. A definite diurnal fluctuation in the oxygen concentration is apparent. There was also a minor fluctuation in the temperature. The oxygen saturation which had been above 100 per cent during the day, dropped below this figure at approximately 9 PM. It continued dropping to a low of 77 per cent at 6:50 AM. This was followed by an increase in oxygen saturation, which exceeded 100 per cent before noon.

The calibration reservoir was equilibrated with atmospheric air by shaking once, at 3:00 PM on May 25. At approximately 6 o'clock PM a N.W. wind accompanied by about a 20 F drop in air temperature apparently caused a density layering of the water in the calibration carboy. During the calibratory procedure, the polarographic probe analyzed water about midpoint in the carboy depth; the chemical (Winkler) samples were withdrawn by siphoning from the very bottom of the 20 liter carboy. It is believed that this procedure produced the difference observed in the chemical analysis and the polarographic readings at the 9:00 and 12:00 PM calibrations. At these times, the difference between the two methods was approximately 3 per cent; all the other calibrations checks averaged less than 0.5 per cent difference.

The results of this study in the field and preliminary laboratory trials showed that calibration of the system every six hours would be sufficient for general purposes. It is evident that for optimum performance, one should mix the calibration reservoir previous to each calibration to achieve a homogeneous oxygen saturation and temperature, and the battery D.C. input voltage to the recorder amplifier should be stabilized. In our apparatus, stabilization has been accomplished by interposing a trimming rheostat and a voltmeter between the battery and the recorder amplifier.

Difficulties, marked by low cell voltage and current, were experienced from time to time with the Jarrell-Ash instrument which is an integral part of the field assembly. This occurred if the instrument was turned on when there was no water flow past the transducer. Complex oxygen reduction reaction reactions at the platinum cathode may be the cause of this difficulty (Lingane, 1961; Sawyer and Interrante, 1961). This difficulty can be avoided if the accessory stirrer is used.

### 3. Fabrication of Galvanic Cells for Special Uses

Galvanic cells of the type proposed by Mancy, Okun, and Reilly (1962) have been fabricated in the laboratory. At 20 C two electrodes produced approximately 10 amps, and a larger one 50 amps. This 50 amp output was adequate to meet the requirements of a portable recording microammeter. The performance of the smaller electrodes indicated that they could be used to replace the Beckman oxygen analyzer in the flow-through respirometer. However, the larger electrode will have to be redesigned to shorten its response time before it is serviceable for field use.

## D. DISCUSSION AND SUMMARY

### 1. Polarographic Flow-Through Respirometer.

The polarographic flow-through respirometer which has been developed (Fig. 1; Plates 1 and 2) will conveniently measure oxygen utilization rates of about 50 to 1000 micro grams per hour from an aqueous environment. With the immature aquatic insects used, this means that a range in wet body mass from about 0.3 to 6 grams can be accommodated. The lower or upper limits of this range can be extended by making the lumen of the respiratory chamber and the connecting tubing appropriately smaller or larger.

A change in the rate of oxygen metabolism of 5 per cent is readily discernable to the eye from the oxygen saturation tracing of the chamber effluent. Smaller changes of the order of 0.5 per cent of the metabolic rate are definable through analysis of areas under the tracing with a polar planimeter. Plate 4 illustrates the sensitivity of the method. In the tracing from the 850 mg dragonfly naiad the portion of the recording reflecting the oxygen saturation in the respiratory chamber effluent "X" had a negative slope, downwards to the right, during periods of the naiad's respiratory activity, and a positive slope, upwards to the right, during the period of respiratory inactivity. Respiratory activity in this case was evidenced by rapid, rhythmic contractions of the naiad's abdomen. Such illustrative tracings were only obtained when the subjects' orientation in the respiratory chamber remained essentially the same for ten minutes or longer.

The respirometer could easily be modified into a "closed circulating pipe" (see Fig. 1) by inserting the tubing on the discharge side of the pump "K" back into the reservoir "B", sealing reservoir "B" from the atmosphere, and eliminating the aeration device "A". The "closed circulating pipe" configuration would permit a more exhaustive study of the effects of flow velocity upon respiration rates (Jaag and Ambuhl, 1963).

## 2. Portable Equipment for Continuously Recording

### Dissolved Oxygen Saturation and Temperature.

With proper maintenance and operational procedure, the portable field equipment for recording dissolved oxygen saturation and temperature (Plate 5 and Fig. 7) which has been designed and assembled will give a recording of the oxygen saturation which will not drift from its calibration more than  $1\frac{1}{2}$  per cent in 6 hours, and a temperature tracing which will drift less than 0.2 C during the same interval of time. The equipment can be used in flowing or stagnant water.

The recording, 5-inch strip chart microammeter, with its accessory voltage regulator and clockwork activated double-pole double-throw micro switch, is readily adaptable to the "in situ" continuous recording of other transduceable water quality parameters, such as flow velocity, turbulence, illumination, and pH. With a more elaborate cyclic switching mechanism, the various water quality sensing and transducing units can be used in concert to record sequentially the fluctuations occurring in a natural aqueous environment.

## E. BIBLIOGRAPHY

- Jaag, O. and H. Ambuhl, 1963. The effect of the current on the composition of biocoenosis in flowing water streams. Intern. J. Air Water Poll. Pergamon Press 7:317-330.
- Lingane, J. J., 1961. Chronopotentiometric study of oxygen reduction at a platinum wire cathode. J. Electroanal. Chem. 2:296-309.
- Mancy, K. H., Okun, D. A., and C. N. Reilley, 1962. A galvanic cell oxygen analyzer. J. Electroanal. Chem. 4:65-92.
- Sawyer, D. T. and L. V. Interrante, 1961. Electrochemistry of dissolved oxygen. II. Reduction of oxygen at platinum, palladium, nickel, and other metal electrodes. J. Electroanal. Chem. 2:310-327.

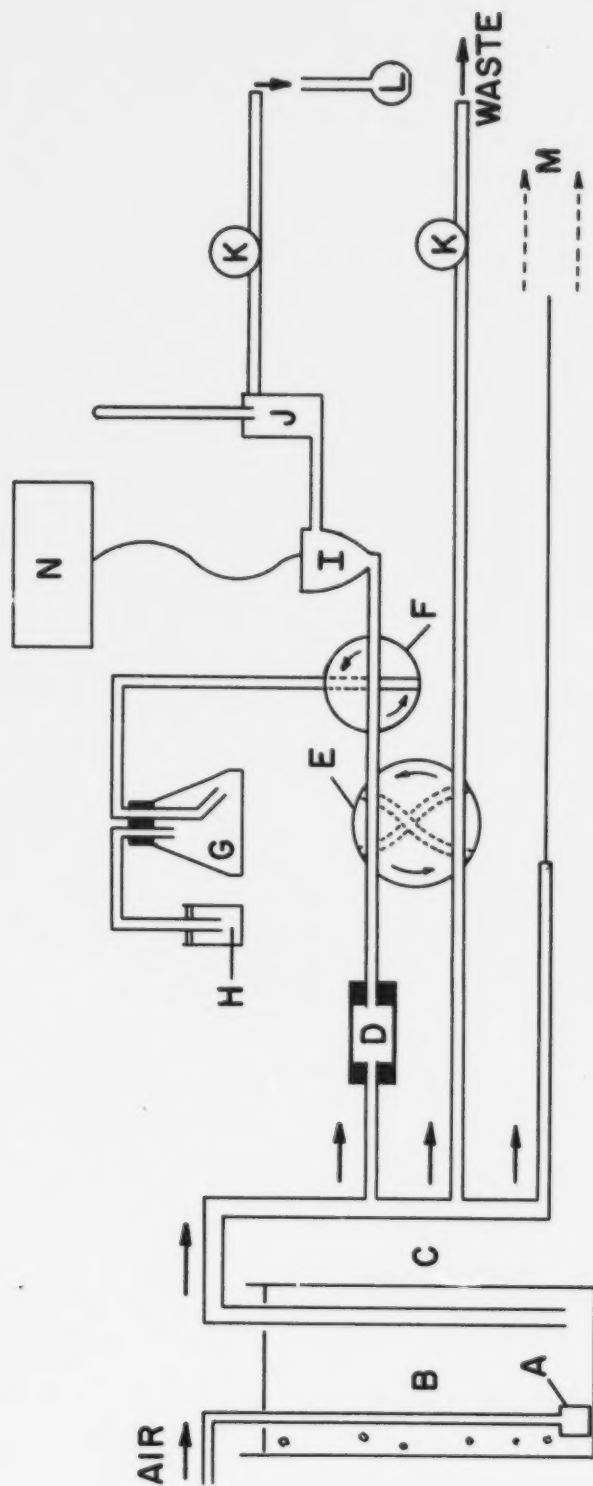


Figure 1 Schematic Flow Sheet: Flow-Through Respirometer Provided with an Oxygen Transducer.

Key:

- |                                             |                                                           |
|---------------------------------------------|-----------------------------------------------------------|
| A. Aeration Stone.                          | K. Variable Speed Harvard Precision Peristaltic Pump.     |
| B. Reservoir.                               | L. Flask for Flow Measurement.                            |
| C. Water Supply Manifold.                   | M. Outlet for pH and Chemical D.O. Measurement (Winkler). |
| D. Chamber.                                 | N. Amplifier and Readout.                                 |
| E. Four-Way Stopcock for Span Adjustment.   | O. Beckman Expanded Scale pH Meter.                       |
| F. Three-Way Stopcock for Zero Adjustment.  | P. Beckman D.O. Analyzer.                                 |
| G. Sulfite Reservoir                        | Q. Beckman Potentiometric Recorder.                       |
| H. Sulfite Solution, Air Trap (Oil Sealed). | --- Line at E Indicates Span Adjustment Position.         |
| I. Oxygen Transducer.                       | --- Line at F Indicates Chemical Zero Position.           |
| J. Mercury Thermometer.                     | --- Line at M Indicates Two Alternative Positions.        |

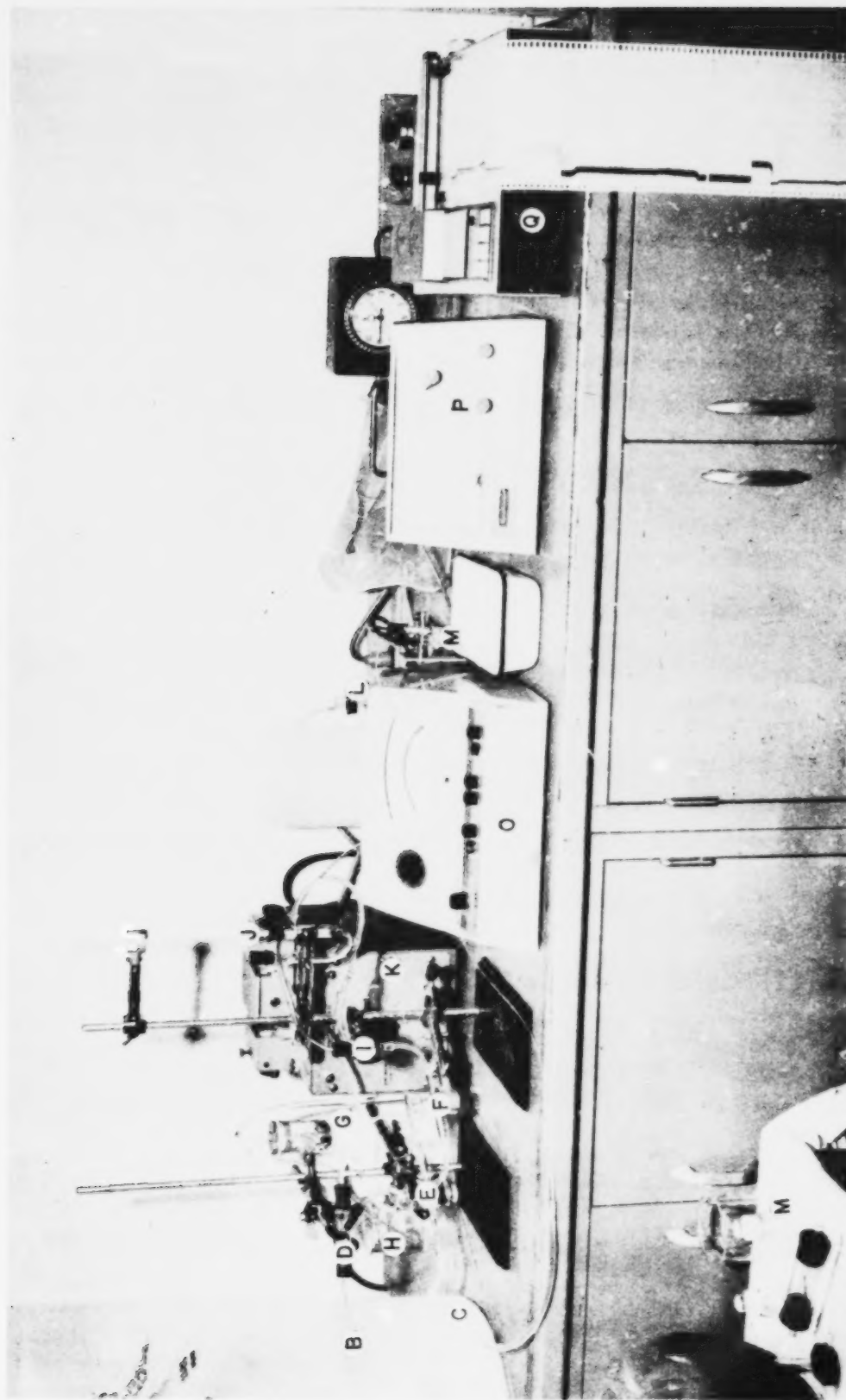


Plate I General View of Polarographic Flow-Through Respirometer.  
(See Figure I for Key)

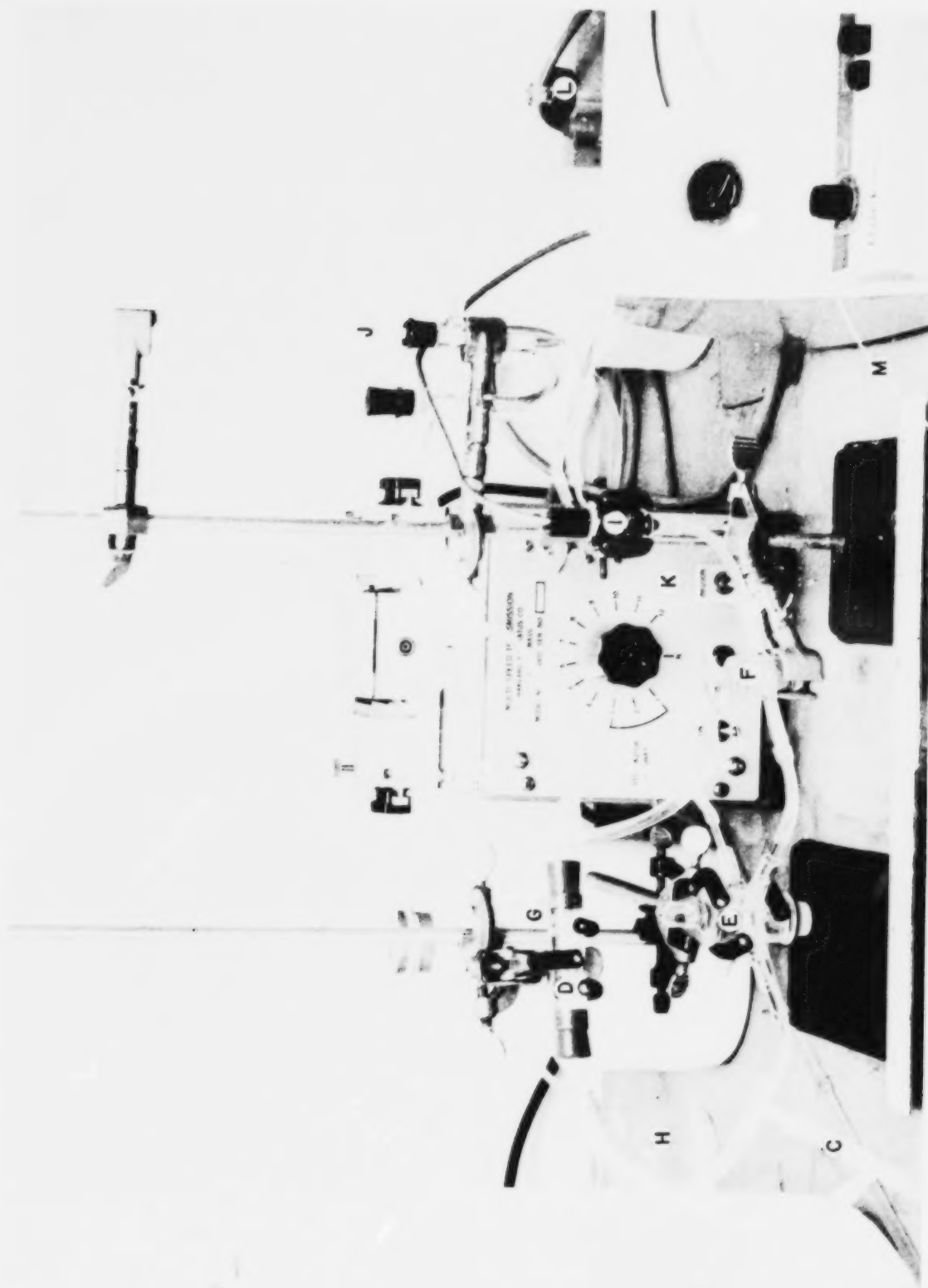


Plate 2 Detail View of Polarographic Flow-Through Respirometer Exclusive of Amplifier and Recorder.  
(See Figure 1 for Key)

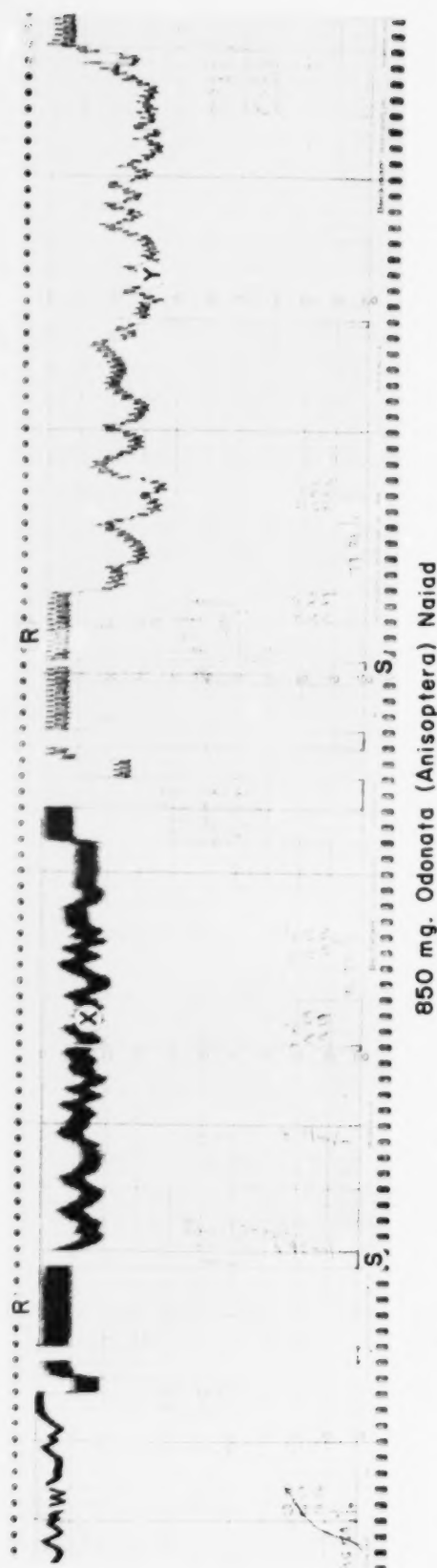
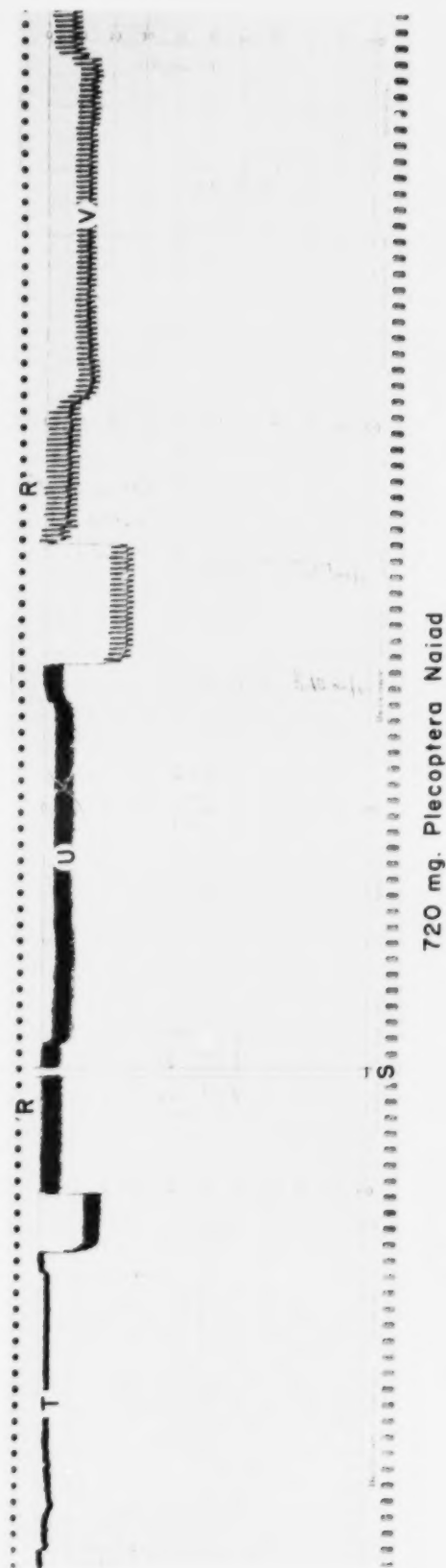
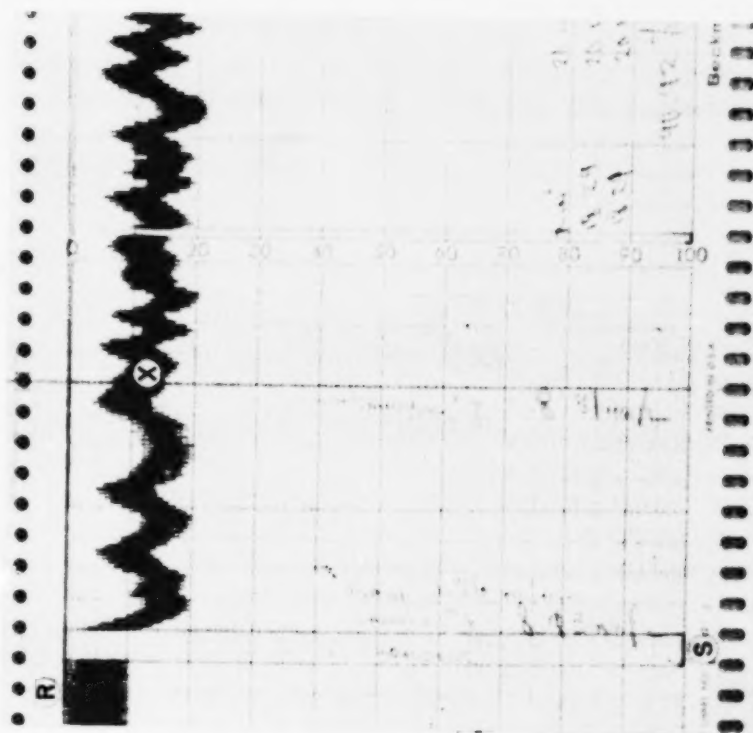
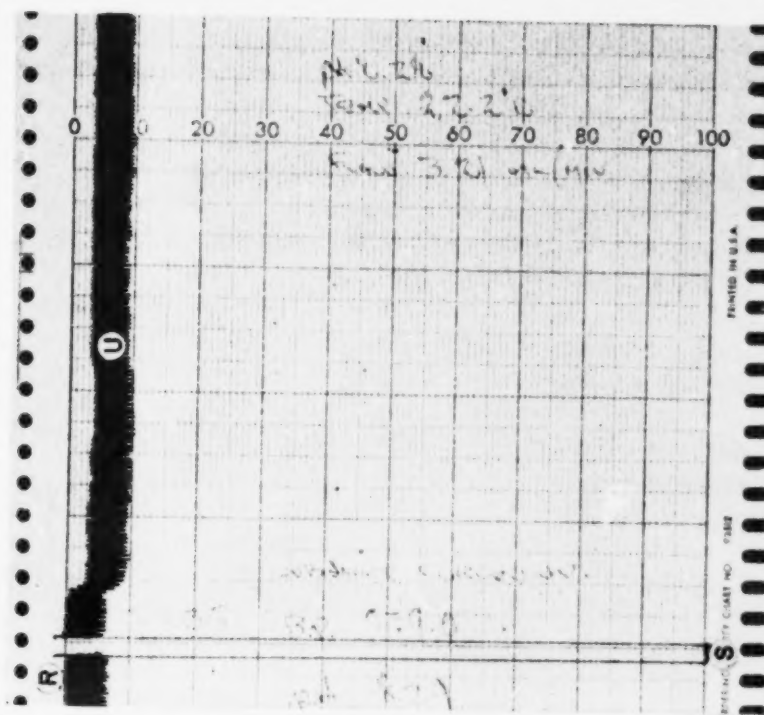


Plate 3 Typical Recordings Obtained with the Polarographic Flow-Through Respirometer.

Key: R- Recorder Span Adjustment U- Flow Rate = 3.21 ml./min. X- Flow Rate = 3.26 ml./min.  
 S- Recorder Zero Adjustment V- Flow Rate = 1.30 ml./min. Y- Flow Rate = 1.34 ml./min.  
 T- Flow Rate = 6.36 ml./min. W- Flow Rate = 6.55 ml./min.



850 mg. Odonata (Anisoptera) Naiad



720 mg. Plecoptera Naiad

Plate 4 Enlarged Sections of Plate 3 Showing Respiration Patterns

Key: R- Recorder Span Adjustment U- Flow Rate 3.21 ml./min.  
S- Recorder Zero Adjustment X- Flow Rate 3.26 ml./min.

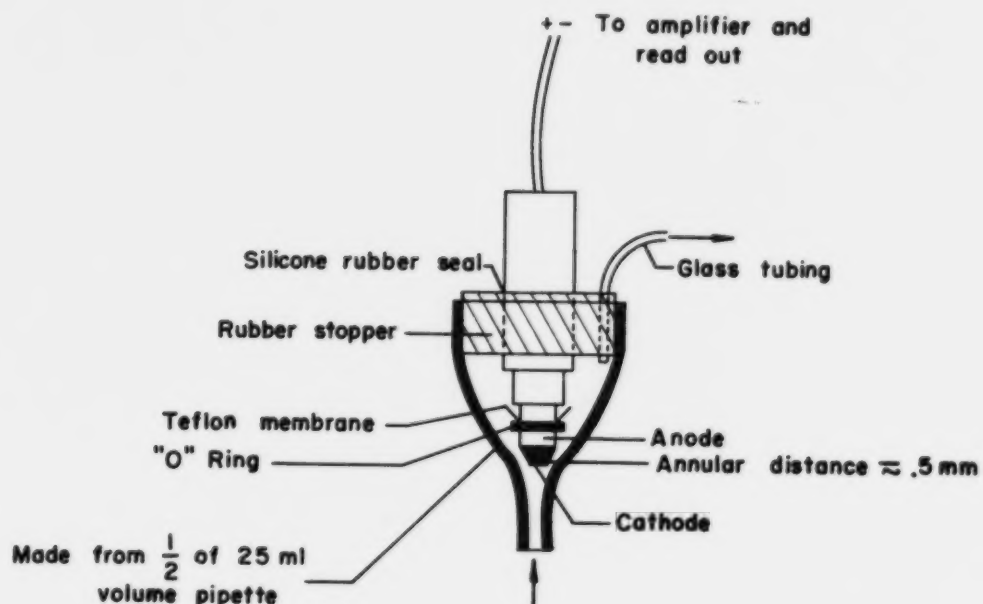


Figure 2 Oxygen Transducer—Flow Detail

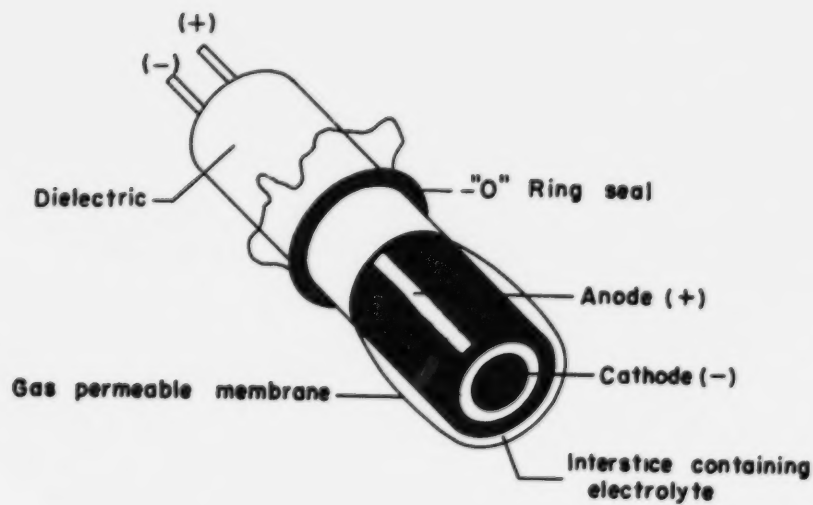
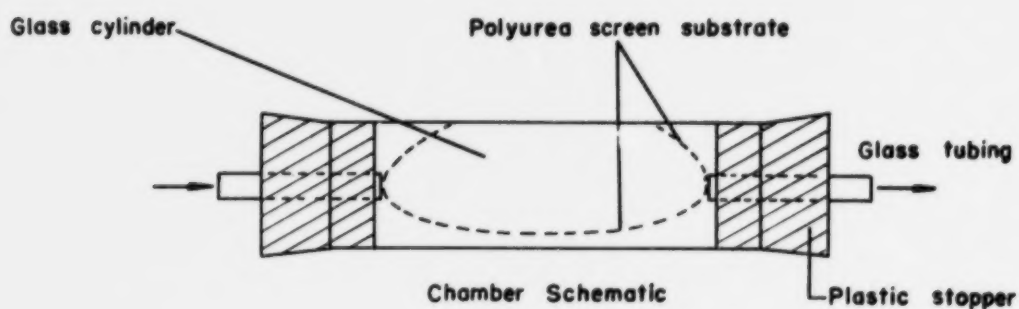


Figure 3 Basic Features "Covered" Polarographic Oxygen Sensor



|     | Chamber Dimensions    |         |                       |
|-----|-----------------------|---------|-----------------------|
|     | C. S. A.              | Length  | Volume                |
| S-2 | 5.44 cm <sup>2</sup>  | 5.90 cm | 32.10 cm <sup>3</sup> |
| F-2 | 2.165 cm <sup>2</sup> | 5.80 cm | 12.56 cm <sup>3</sup> |

Figure 4 "Flowing" Respirometer Subject Chamber Detail

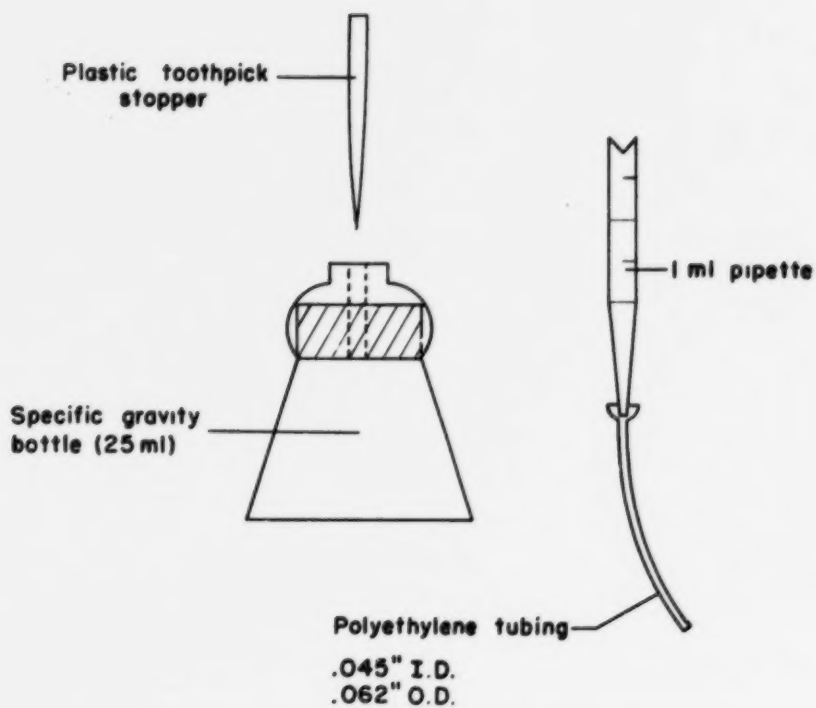
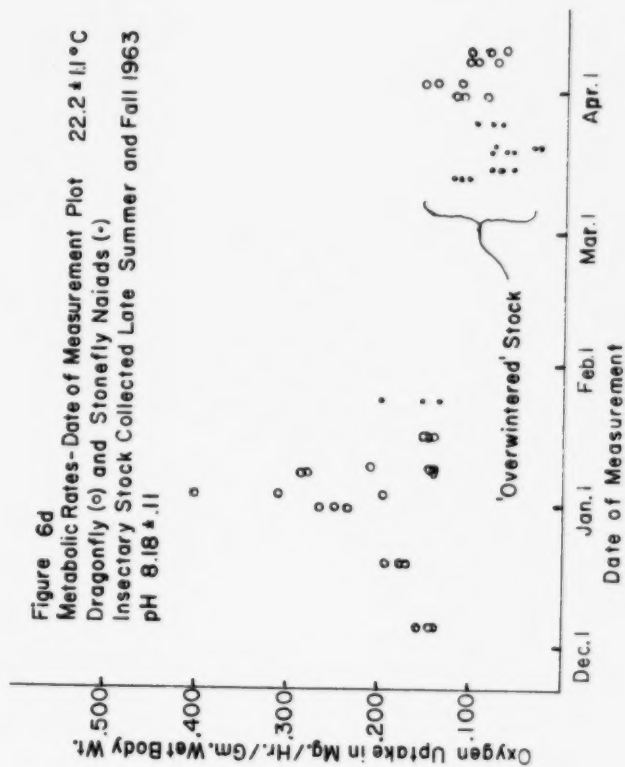
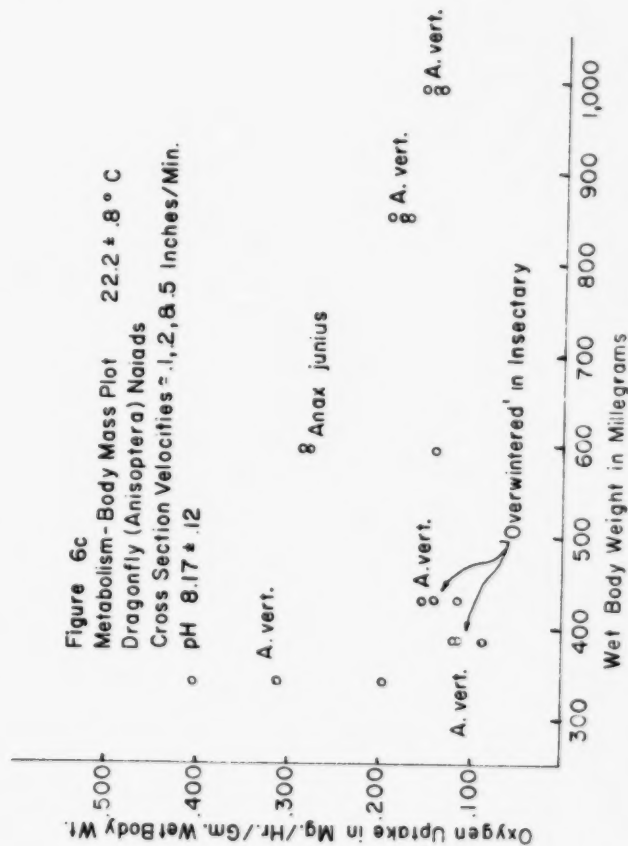
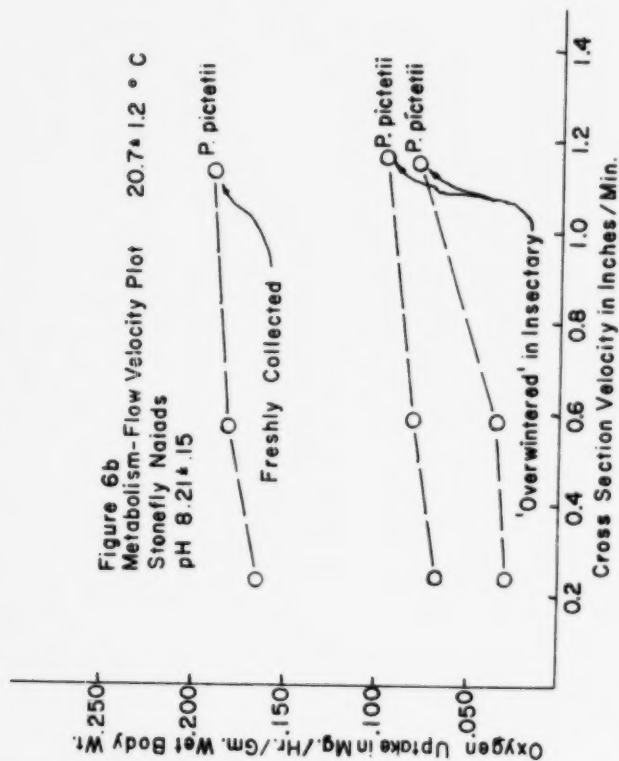
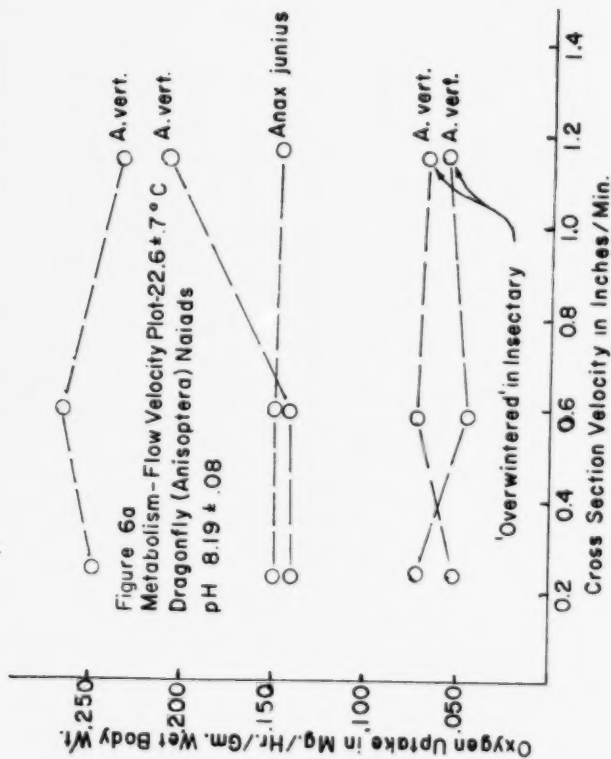


Figure 5 "Micro" Winkler Detail



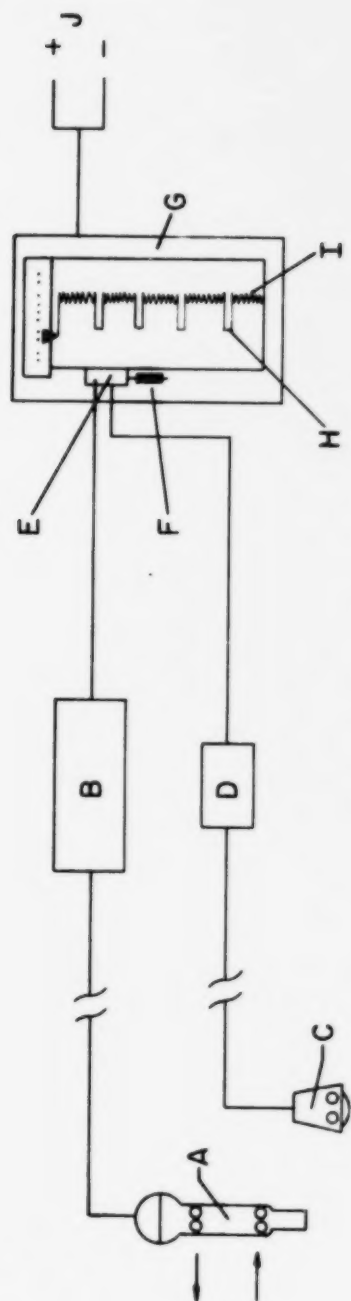


Figure 7 Schematic Diagram: Portable Oxygen Monitor

- Key:
- A. Oxygen analyzer immersion stirrer containing oxygen transducer (Jarrel-Ash).
  - B. Oxygen analyzer amplifier and readout circuitry (Jarrel-Ash).
  - C. Electronic thermometer thermistor capsule.
  - D. Electronic thermometer bridge circuit and battery.
  - E. Double pole-double throw microswitch.
  - F. Actuating cam for microswitch.
  - G. Recorder (Esteline Angus portable).
  - H. Temperature record (records during 12% of cycle).
  - I. Dissolved oxygen saturation record (records during 88% of cycle).
  - J. 12 volt D.C. input to amplifier.
  - K. Temperature correction unit.
  - L. Mercury thermometer.



Elk River at Birch Island Lagoon Study

**Overnight Monitor of Dissolved Oxygen Saturation and Temperature**

### Lagoon Characteristics:

140 Ft.

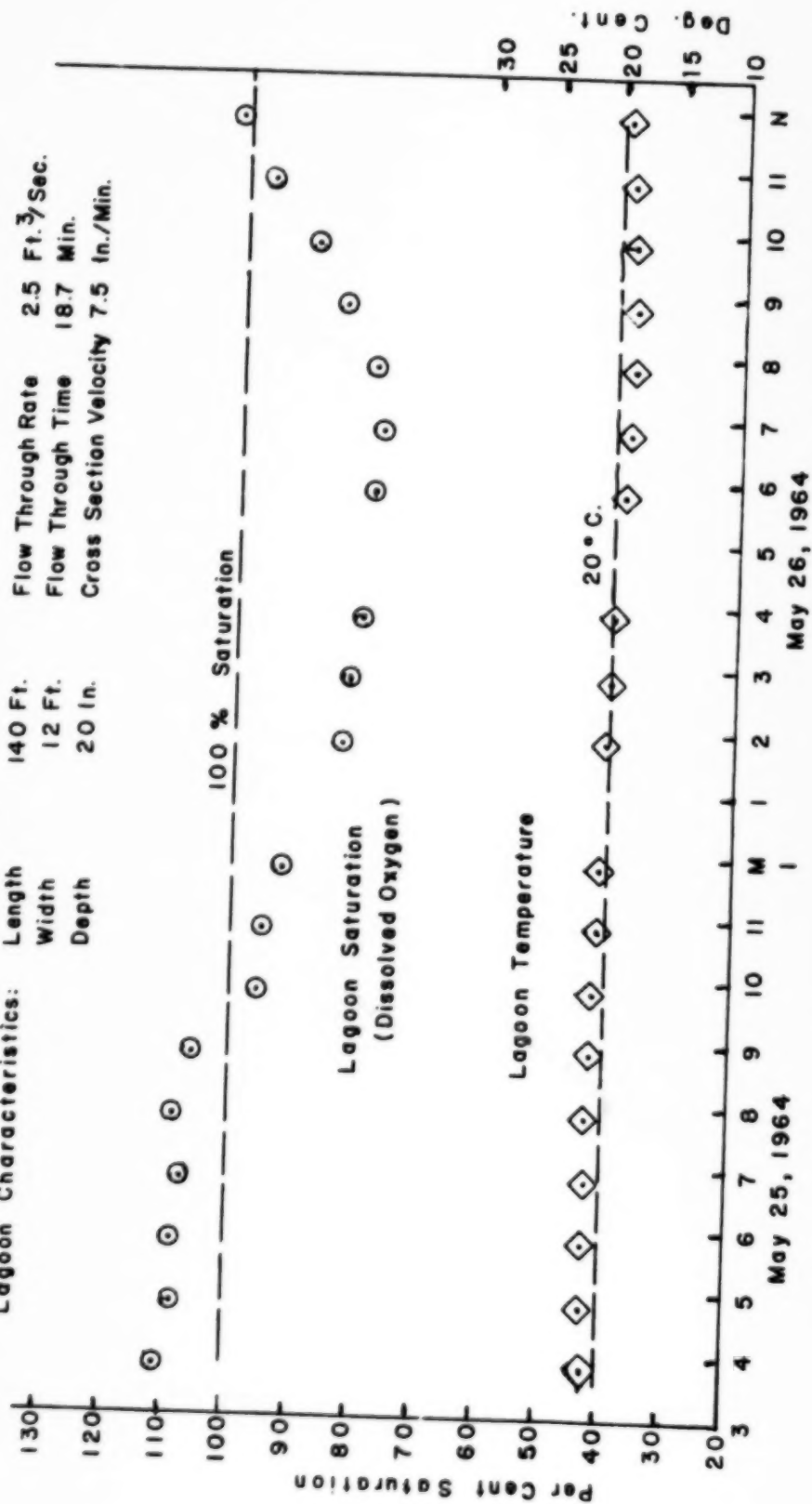
12 F4

2016

**Flow Through Rate** 2.5 Ft.<sup>3</sup>/Sec.

Flow Through Time 18.7 Min.

**Cross Section Velocity 7.5 In./Min.**



### III. WARBURG METHOD AS USED IN THIS INVESTIGATION

#### A. OBJECTIVES

The studies to date have been primarily concerned with: (1) the determination of the individual oxygen consumption rates of typical benthic immature insects in an attempt to evaluate the oxygen requirements of each accompanied with a few studies using multiple specimens, the effect of temperature, and the effect of the use of a screen substratum in the respiratory flasks; (2) the comparison of the oxygen consumption rates of the different orders and families and how these rates may be related to the index-organism classification; and (3) a comparison of the Warburg method and the electro-chemical flow-through method using the same individuals or members of the same species. Accompanying these studies has been: (1) the effect of the length of the equilibrium period on the oxygen consumption rate; (2) identification of the insects used; and (3) putting on punch cards, the data obtained from the oxygen consumption studies.

#### B. METHODS AND PROCEDURE

The method followed the general plan as outlined in Umbreit, et al (1964), using 3 ml deionized water in 15 ml flasks with 0.2 ml of 10 per cent KOH and folded filter paper in the well. In the earlier experiments screens, which would serve as a substratum for the insects, were not used in the flasks; after November 1963, a screen was added to each flask including the thermobarometer flask. Flasks were shaken at 88 times per minute at an excursion distance of 3 cm with the 9-station, circular, Aminco Warburg apparatus in an unlighted temperature-controlled room. Insects were allowed to equilibrate for  $\frac{1}{2}$  to 1 hour before readings were started. Readings were taken each hour for a period of 4 hours. Oxygen consumption is expressed in  $\mu\text{l O}_2$  per gram wet weight per hour. The hourly consumption rate and occasionally the 15-minute rate was determined in some cases. With the exception of several examples of the hourly rate, the oxygen consumption rate expressed in this report is an average of the 4 hours. In the experiments for which the hourly rate is illustrated, eight specimens were tested in each experiment (a full run for our 9-station Warburg apparatus). The rates obtained each hour were computed, the eight then averaged, and the standard deviation computed. Four experiments were done using Pteronarcys pictetii (Plecoptera), three for a four-hour period and one for a six-hour period; two experiments, each for a four-hour period, were done using Limnephilus consocius (Trichoptera).

The data necessary for computation of the hourly rate is available and may be used at some later date, since it may be desirable to allow a longer equilibratory period than  $\frac{1}{2}$  to 1 hour. The wet weight of the insects was determined by weighing them on a Sartorius balance in closed weighing bottles containing a little water. Excess moisture was removed from the insects by blotting them with filter paper before the weighing. Volume of the insects was determined by the replacement of water in volumetric containers; a Babcock bottle was used for smaller insects and a blood sedimentation tube for larger ones.

Individual insects were used in most experiments; in those cases in which the particular insect was minute, multiple specimens were used because the Warburg apparatus has insufficient sensitivity to measure the hourly consumption rate of an individual specimen. Except for the Macronemum zebratum, the oxygen consumption rates of the Trichoptera with cases were done without removing the larvae. The cases of M. zebratum are incomplete if removed from the rocks. We decided that the removal of the other Trichoptera from their cases might injure the larvae and would be an unnatural state for them. The oxygen consumption rate was calculated on the basis of the weight of the larvae. An empty case of the particular species under test was not added to the thermobarometer flask in these experiments; this probably would have been desirable and will be practiced in future experiments because these may have an oxygen consumption of their own.

Most of the insects were collected from rather rapidly flowing rivers in Minnesota. The Neuroptera were from the Apple River, Wisconsin, *M. zebratum* (Trichoptera) from the St. Croix River, *Simulium* (Diptera) and Odonata (Zygoptera) from creeks in the Minneapolis and St. Paul area. The other insects came from the Elk River. The insects were kept in aquariums containing water and rocks from the original or a similar habitat. Light was provided during at least part of the day. Some of the aquariums were maintained at approximately 5 C, and others at 20 C. Previous to spring 1964, the water was aerated or circulated by pumps or air lines and filtered through charcoal and glass wool about once a month. The aquariums are now equipped with bottom filters of coarse sand; the water, after passing through the sand, is filtered through activated charcoal. With this procedure, algae grow but have not produced blooms or excessive coating on rocks and other surfaces.

The insects were brought to approximately 20 C at least 2 hours before the beginning of an experiment and conditioned to deionized water by progressively increasing its proportion to the aquarium water. Some of the insects, such as the Odonata which burrow into the sand of the aquarium, were lightly brushed to rid them of accumulated debris adhering to the hairs and body.

The main emphasis of the oxygen consumption studies was given to experiments carried out at 20 C; however, in some instances studies were made at a series of temperatures. Suggestions of Holman (1962) based on Fisher and Yates table of *t* were used as a guide for the number of individuals to be used in the experimental work. On this basis, 32 individuals of each species was chosen as a beginning number; larger numbers would be used if extensive gaps in the range occurred. With this number, the 99 per cent confidence level is about 6 per cent greater than that for the normal curve. This number of determinations has not been carried out, as yet, for each genera or species since some are more readily available than others; it is hoped that these series can be completed by future collections and respiratory studies.

In the manometric method, since stopcock leakage is particularly important, the precautions suggested by Ludwig, et al (1951) were practiced. It involved the following procedure: (1) the stopcock was removed, and it, the stopcock barrel, and the connection for the respiratory flask was wiped free of grease with tissue of a rather firm surface; (2) they were then cleaned with tissue wetted with xylol; (3) the stopcock was greased by applying thin longitudinal streaks of Lubro-Seal on three sides of the plug between the bores; (4) the plug was warmed gently in a blue bunsen flame, inserted in the barrel with the vertical bores coinciding, and pressed in firmly until the three zones of lubricant coalesced; (5) three dots of lubricant were applied to the manometer joint fitting into the respiratory flask; (6) the top of the flask was warmed gently in the blue flame and pressed unto the joint; (7) both the stopcock and respiratory flask joining were examined to see that they had a smooth appearance over the entire surface; (8) in the event that uneven areas were present, the entire process of cleaning and greasing was repeated; (9) any lubricant which flowed into the bores of the stopcock was removed with a syringe and long needle.

Ludwig, et al (1951) also caution that the respiratory flasks must be strictly clean. The flasks were cleaned in the following manner: (1) the grease was wiped from the mouth of the flask and from the stopper with tissue, followed by wiping with tissue moistened with xylol, and then one wetted with alcohol; (2) the flasks and stopper were scrubbed with warm water and Lava soap, followed by five rinses with tap water and three with distilled water; (3) the flasks were filled with dichromate solution and placed on a steam bath for two hours; (4) the dichromate was poured out and the flasks and stoppers rinsed twelve times with tap water five times with distilled water, and two times with deionized water; (5) the flasks and stoppers were dried in a 140 C oven for one hour; (6) the flasks were stored in an inverted position in a closed cabinet.

## C. RESULTS AND DISCUSSION

### 1. Oxygen Consumption Rates of Immature Aquatic Insects

Oxygen consumption rates have been determined by the Warburg method on immature aquatic insects of the orders Coleoptera, Diptera, Ephemeroptera, Neuroptera, Odonata, Plecoptera, and Trichoptera. The results obtained from 679 insects of these orders are presented in Table 1 and Figures 1-4. The figures show the individual oxygen consumption rates, while averages are given in the table. In the figures, the symbols indicate the following: (a) open; individual specimens in respiratory flasks with screens; (b) black; individual specimens in respiratory flasks without screens; (c) with two crossed lines; multiple specimens in respiratory flasks without screens; and (d) with one line; multiple specimens in respiratory flasks with screens. Most of the determinations were made at 18-21 C. Usually little variation occurred in the rates at this temperature range, and some of the variance could have been due to differences in size of the test specimen. Smaller specimens have been found by previous workers to have higher consumption rates than larger specimens of the same species. We have not determined the rates on sufficiently large samples of each size of each species, but this relationship of size and oxygen consumption rate was shown by Macronemum zebratum (Trichoptera) (see Fig. 6a and 6c).

In conditions of marked temperature rises, the oxygen consumption rate also increased (Fig. 1-4; Table 1), especially when no screen substratum was used. With a screen substratum in the flasks, the rates were lower than without such supports. These screens provided a substance for the insects to cling to, and thus provided a more normal situation. In nature most of these insects are found on rocks, the sand, or aquatic plants. In the laboratory aquariums, which were supplied with strips of the same screening for emerging insects, the early immatures often used these strips in preference to rocks.

A few studies have been made using multiple specimens; most of these have been on genera where the size of the insect made it necessary to use more than one specimen. These oxygen consumption rates have been somewhat higher than for individual specimens. This may have been due in part to the smaller sizes of the specimens; however, insects were more active when tested in groups, and this increases the rate.

### 2. Comparison of the Oxygen Consumption Rates and Their Relationship to the Index-Organism Classification

The average comparative oxygen consumption rates of various individual immature aquatic insects at 20 C in respiratory flasks containing a screen substratum are shown in Figure 5. These consumption rates, the average weights, and their respective standard deviations are given in Table 2. Further comparisons of insect groups and genera are given in Figure 6c and Table 1. These oxygen consumption rates may be related to the index-organism classification in Fair and Whipple (1948). This list is incomplete, especially for North American species; therefore, several other survey references are used in addition (Gauvin and Tarzwell, 1952; Sinclair, 1964).

Only a limited number of tests have been done on the Coleoptera, but they appear to have a high oxygen consumption rate. The rates may be higher than those which occur in nature; our tests were made in flasks without the screen substratum. Also, in tests with Elmidae and Helodidae, multiple specimens were used because of their minute size. Fair and Whipple list no immature Coleoptera in their tabulation, however, Sinclair found Elmidae mostly in clean water zones and Gauvin and Tarzwell found species of this family only in the clean water zone preceding the septic zone.

They did not find them in the clean water zone following the zone of recovery. As a group, the Coleoptera may not be as useful indicators of water quality as some of the other orders. Approximately 20 families have aquatic larvae, but only 6 and a small part of another use dissolved oxygen. The remaining aquatic families have functional spiracles by which they obtain atmospheric oxygen, specialized structures for piercing plants, or burrow within aquatic plants by which they obtain intercellular air. The Hydrophilidae used in our tests have lateral abdominal gills and use dissolved oxygen. The Elmidae apparently accomplish their respiration by means of the retractile gills situated in the caudal chamber (Leech and Chandler, 1956). The Dystiscidae and Helodidae use atmospheric oxygen.

In our studies, the Diptera had considerable variation in their consumption rates; the Simuliidae had a high rate and the Tipulidae a low one (Fig. 1). The rates for the Simuliidae may have been higher than normal because multiple specimens were used to compensate for their small size and because the flasks did not have a screen substratum. In nature, the larvae attach to rocks or plants. When compared with the other orders, the oxygen consumption rates of Tipulidae were always lower (Fig. 5 and 6c). Edwards (1958), using Chironomus riparius in the Warburg apparatus, obtained consumption rates similar to those in our study on Chironomus sp. (Tables 1 and 3). The Simuliidae and Chironomus (Tandipedidae) obtain their oxygen from the water by cutaneous respiration; however, the hemoglobin in some species of the latter family may act in oxygen transport at very low oxygen concentrations and aids in their recovery from periods of oxygen lack (Walshe, 1950). The Tipulidae, Rhagionidae, most of the Culicidae, and many other dipteran larvae have posterior respiratory spiracles by which they obtain atmospheric oxygen. Chaborus larvae have air bladders, and the gases in these come into diffusion equilibrium with any gas mixture in the surrounding water (Wigglesworth, 1950). Dipterous larvae with open spiracles may have to depend on dissolved oxygen when the surface tension of the water is reduced by pollution. For example, Culicidae larvae are unable to hold to the surface when the tension is reduced from the normal 70 dynes per cm<sup>2</sup> to about 27-36 dynes (Wigglesworth, 1950).

Although the oxygen consumption rate of the families of the Ephemeroptera varied somewhat (Fig. 2 and 5), they had higher rates than the other orders. The high oxygen consumption rate of this order corresponds to their index-organism classification. In the Fair and Whipple tabulation, the Ephemeroptera are classified in the clean water zone for most part. Caenis fumosa is listed as having been found in the recovery zone, and Gaufin and Tarzweil found Caillibaetis sp. in this same zone in their study. As yet, these genera have not been tested by us. Fox, Simmonds, and Washbourn (1935) compared the oxygen consumption rates of Caenis sp. and Ephemerella ignita; the rates averaged 290 and 950  $\mu$ l O<sub>2</sub>:gm dry weight:hr, respectively. The rates Fox, et al (1935) obtained for Ephemera vulgata and Ephemera danica probably would average near the figure obtained by us for Ephemera sp. if the temperature and dry and wet weight differences are considered (Tables 1 and 3). The dry weight is about 25 per cent of the wet weight (Heilbrunn, 1952). The Ephemeroptera usually obtain their oxygen from the water by respiring through gills. Some species can survive in highly oxygenated water without their gills, and under these conditions obtain their oxygen needs by cutaneous respiration. (Wigglesworth, 1950).

The Neuroptera (Chauliodes) used in our tests have lateral respiratory gills enabling these insects to use dissolved oxygen; however, they also have a pair of posterior tubes with spiracles that may enable them to come to the surface for oxygen. In addition, their oxygen requirements appear to be low (Fig. 5). Fair and Whipple list Sialis lustaria (Family Sialidae) as being found in the recovery zone. Gaufin and Tarzweil found Sialis and Corydalis cornutus (Family Corydalidae) only in the clean water zones. The Chauliodes and Corydalis have an aquatic life period of two to three years; hence, the presence of mature larvae in a body of water would show that the water quality was sufficient for their needs for an extended period.

Although additional determinations with screens in the flasks are needed to complete the studies on the Odonata, our available data indicate that they have a moderate oxygen consumption rate (Fig. 5 and 6c). In the Fair and Whipple tabulation, the Odonata for most part are listed in the clean water zone. The naiads of the suborder Anisoptera obtain oxygen by passing water in and out of a rectal chamber which has an elaborate system of tracheal gills. Passage of the gas to the underlying tracheoles is by simple diffusion; the partial pressure of oxygen in the tracheal system of the living naiad is less than that of water (Wigglesworth, 1950). The naiads of the suborder Zygoptera have lamellate caudal gills by which they ordinarily obtain their oxygen. The general body surface is also able to absorb oxygen, but is not as efficient as the gills (Wigglesworth, 1950). Many of the naiads require two to four years to reach maturity (Smith and Pritchard, 1956). Three specimens on which repeat determinations were made after the passage of  $2\frac{1}{2}$  to 3 months had a lowered oxygen consumption rate. These specimens were kept in an aerated 20 C aquarium which was not filtered continuously. The naiads were fed mosquito larvae. Although the weight of all three specimens increased during the period, it appears that some of the decrease in the oxygen consumption rate was due to storage in the laboratory. The decreases in the consumption rate and increases in weight were as follows:  $97\ \mu\text{l}$  (179.7 mg) to  $53\ \mu\text{l}$  (211.2 mg);  $125\ \mu\text{l}$  (678.7 mg) to  $54\ \mu\text{l}$  (724.1 mg); and  $132\ \mu\text{l}$  (344.6 mg) to  $66\ \mu\text{l}$  (378.4 mg).

In Fair and Whipple's tabulation, six families of Trichoptera are listed as having been found in the recovery zone. In our studies, their average oxygen consumption rate was below that of the Ephemeroptera (see Fig. 5), which would appear to fit their index-organism classification. The determinations on Macronemum zebratum may be somewhat higher than that found in nature since they were removed from their cases. Storage of the M. zebratum in the laboratory appeared to lower their oxygen consumption rate especially when they were held for several months (see Fig. 6a). These had been collected during late fall and held in circulating stream water in open fiberglass bins in a 5 C room; the water was filtered through charcoal and glass wool about once a month. Some of the Trichoptera have respiratory gills, but evidently the larvae use cutaneous respiration for most part. Morgan and O'Neil (1931), utilizing the Winkler method, found that amputation of the tracheal gills on M. zebratum reduced oxygen consumption slightly and sometimes not at all. Therefore, they concluded that the gills are only accessory organs; the oxygen intake occurs through the body integument. Normal larvae eliminated  $\text{CO}_2$  more rapidly than larvae without gills.

### 3. Comparison of the Warburg Method and the Electro-Chemical Flow-Through Method

Results in which the oxygen consumption rates of individual specimens were compared, using the flow-through method and the Warburg method, are shown in Table 4. Generally, the flow-through method gave slightly higher rates; however, temperature differences in some cases must be considered. Additional comparisons are needed. Edwards and Learner (1960), in their comparative study of the Warburg apparatus and the dropping mercury electrode method utilizing a flow-through respiratory chamber, found that the oxygen consumption of Asellus aquaticus (Malacostraca: Isopoda) was significantly higher ( $P < 0.001$ ) in the polarographic respirometer than in the Warburg. A rate of between 35 and 75 per cent higher was obtained with the polarographic method. In an earlier paper, Edwards (1958) stressed the need for comparative studies, using different respirometric systems.

#### 4. Accompanying Studies

Examples of the experimental results in which hourly consumption rates were computed and averaged for eight specimens, in order to determine the effect of a longer equilibratory period, are shown in Figure 6b. The results of four experiments, three over a four-hour period and one over a six-hour period, using Pteronarcys (Plecoptera) showed a definite decrease in the average consumption rate and the standard deviation. Limnephilus (Trichoptera) gave results similar to those usually obtained when hourly rates were computed.

The references used in identifying the insects tested in these oxygen consumption studies are listed in the bibliography following this section. The insects were identified to family, genus, or species, depending upon the completeness of available identification keys. These vary because: (a) the immature stages of many insects are unknown; (b) some have not been sufficiently characterized to be separable from morphologically similar forms; and (c) the keys are written for the identification of the late larval or nymphal stages and in most cases are not satisfactory for the early instars. For these reasons, it is planned to have the identifications verified and completed by specialists working with particular orders or families of these insects. Putting the data from the oxygen consumption studies on punch cards greatly facilitated the tabulation and comparison of the data, especially as the determinations increased in number.

#### D. SUMMARY

1. Using the Warburg apparatus, oxygen consumption rates have been determined on 679 immature aquatic insects. These are from the insect orders Coleoptera, Diptera, Ephemeroptera, Neuroptera, Odonata, Plecoptera, and Trichoptera. Thirty-two families were represented in these orders. Most of the determinations were done on individual specimens.

2. With a screen substratum in the respiratory flasks, (a) the Ephemeroptera had a high oxygen consumption rate; (b) the Rhagionidae (Diptera), Odonata, Plecoptera, and Trichoptera had moderate rates; and (c) the Tipulidae (Diptera) and the Neuroptera (Corydalidae) had the lowest rates. In general, the consumption rates appear to correspond to the respective index-organism classifications given in the Fair and Whipple (1948) tabulation. Those with high oxygen consumption rates have generally only been found in clean water zones, while those with moderate and low rates have been found in the recovery zones.

3. The oxygen consumption rates of a few immature aquatic insects were determined by the Warburg method and the electro-chemical method utilizing a flow-through respiratory chamber. With the latter method, the rates were usually somewhat higher.

TABLE 1. Average oxygen consumption ( $\mu\text{l/gm/hr}$ ) by immature aquatic insects at various temperatures ( $^{\circ}\text{C}$ ).

|                                 | Total<br>Specimens | Total<br>Tests | 13° | 16° | 17°  | 18°   | 19°   | 20°    | 21°  | 22°  | 27°   | 28° | 30°   | 31°   |
|---------------------------------|--------------------|----------------|-----|-----|------|-------|-------|--------|------|------|-------|-----|-------|-------|
| COLEOPTERA:                     |                    |                |     |     |      |       |       |        |      |      |       |     |       |       |
| Dytiscidae                      | 3                  | 3              |     |     |      |       |       | 560*   |      |      | 875*  |     |       |       |
| Elmidae                         | 15                 | 2              |     |     |      |       | 319** |        |      |      |       |     |       |       |
| Helodidae: Cyphon               | 6                  | 1              |     |     |      |       | 735** |        |      |      |       |     |       |       |
| Hydrophilidae                   | 4                  | 4              |     |     |      |       |       |        |      | 306* |       |     |       |       |
| DIPTERA:                        |                    |                |     |     |      |       |       |        |      |      |       |     |       |       |
| Culicidae: Chaoborus            | 16                 |                |     |     |      |       |       |        |      |      |       |     |       |       |
| Rhyacionidae: A. erix variegata | 5                  | 5              |     |     |      |       | 240*  | 145    |      |      |       |     | 296** | 367** |
| Simuliidae: Simulium            | 35                 | 2              |     |     |      |       |       |        |      |      | 676** |     |       |       |
| Stratiomyidae                   | 12                 | 3              |     |     | 100* |       |       |        |      |      | 23*   |     |       | 350** |
| Syrphidae                       | 1                  | 1              |     |     |      |       |       |        |      |      | 301   |     |       |       |
| Tendipedidae: Chironomus        | 12                 | 1              |     |     |      |       |       | 388**  |      |      |       |     |       |       |
| Tipulidae: Tipulus              | 21                 | 36             |     |     | 74*  | 44*   | 56*   | 47     | 232* |      |       |     |       | 511*  |
| EPHEMEROPTERA:                  |                    |                |     |     |      |       |       |        |      |      |       |     |       |       |
| Baetiscidae: Baetisca           | 16                 | 16             |     |     |      |       |       | 314    |      |      |       |     |       |       |
| Ephemeridae: Ephemera           | 8                  | 8              |     |     |      |       |       | 253    |      |      |       |     |       |       |
| Heptageniidae: Heptagenia       | 1                  | 1              |     |     |      |       |       | 414    |      |      |       |     |       |       |
| : Stenonema                     | 15                 | 15             |     |     |      |       | 467   | 283    | 500  |      |       |     |       |       |
| Leptophlebiidae: Leptophlebia   | 54                 | 30             |     |     |      | 467** |       | 462*   |      |      |       |     |       |       |
|                                 |                    |                |     |     |      |       |       | 485    |      |      |       |     |       |       |
| Polymitarcidae: Ephoron         | 4                  | 4              |     |     |      |       |       | 532*   |      |      |       |     |       |       |
| Potamanthidae: Potamanthus      | 29                 | 29             |     |     |      |       |       | 307    |      |      |       |     |       |       |
| Siphonuridae: Isonychia         | 23                 | 23             |     |     | 244* |       | 412   | 294    | 254  |      |       |     |       |       |
|                                 |                    |                |     |     |      |       | 370   | 440    | 266  |      |       |     |       |       |
| Tricorythidae: Tricorythodes    | 9                  | 2              |     |     |      |       | 370*  |        |      |      |       |     |       |       |
|                                 |                    |                |     |     |      |       |       | 338*** |      |      |       |     |       |       |
| NEUROPTERA:                     |                    |                |     |     |      |       |       |        |      |      |       |     |       |       |
| Corydalidae: Chauliodes         | 16                 | 61             |     |     | 97   | 93    | 92    | 67     | 68   | 58   |       |     |       |       |
|                                 |                    |                |     |     | 169* |       |       | 174*   | 233* | 286* |       |     |       |       |
| ODONATA:                        |                    |                |     |     |      |       |       |        |      |      |       |     |       |       |
| Aeshnidae: Aeshna verticalis    | 14                 | 30             |     | 62* | 43   | 46*   | 65    | 125    | 68   | 52   |       |     |       |       |
|                                 |                    |                |     |     |      |       | 56*   |        | 65*  |      |       |     |       |       |
| : Anax junis                    | 4                  | 9              |     |     |      |       | 59    | 125    | 55   |      |       |     |       |       |
|                                 |                    |                |     | 72* |      |       | 73*   |        | 295* |      |       |     |       |       |
| : Boyeria                       | 1                  | 1              |     |     |      | 98*   |       |        |      |      |       |     |       |       |

TABLE 1 - conti.

| Total                                |       | 13° | 16° | 17°  | 18°   | 19° | 20°  | 21°  | 22°  | 27°  | 28° | 30° | 31° |
|--------------------------------------|-------|-----|-----|------|-------|-----|------|------|------|------|-----|-----|-----|
| Specimens                            | Tests |     |     |      |       |     |      |      |      |      |     |     |     |
| Gomphidae: Gomphoides                | 3     | 3   |     |      |       |     | 99   |      |      |      |     |     |     |
| : Gomphus                            | 3     | 3   |     |      |       |     | 102  |      |      |      |     |     |     |
| Libellulidae: Dorocordila            | 2     | 3   |     |      |       |     |      | 67   |      |      |     |     |     |
| : Leucorrhinia                       | 2     | 2   |     |      |       |     | 94*  | 150  |      |      |     |     |     |
| : Tetragoneuria                      | 28    | 34  |     |      |       |     | 162* | 156* | 201* | 169* |     |     |     |
| Calopterygidae: Calopteryx           | 4     | 7   |     | 239* |       |     |      | 235* |      |      |     |     |     |
| Coenagrionidae: Amphigrion           | 1     | 1   |     |      |       |     |      | 214* |      |      |     |     |     |
| : Ischnura                           | 4     | 4   |     |      |       |     |      | 352* |      |      |     |     |     |
| PLECOPTERA:                          |       |     |     |      |       |     |      |      |      |      |     |     |     |
| Perlidae: Acroneuria abnormis        | 1     | 1   |     |      |       |     |      | 84   |      |      |     |     |     |
| : Acroneuria lycorias                | 1     | 1   |     |      |       |     |      | 169  |      |      |     |     |     |
| : Paragnetina media                  | 10    | 10  |     |      | 109   |     |      | 169  |      |      |     |     |     |
| Pteronarcidae: Pteronarcys           | 49    | 51  | 45  |      | 240*  |     |      |      |      |      |     |     |     |
|                                      |       |     |     |      | 36    | 87  | 133  |      |      |      |     |     |     |
|                                      |       |     |     |      | 152*  |     | 122* |      |      |      |     |     |     |
| TRICHOPTERA:                         |       |     |     |      |       |     |      |      |      |      |     |     |     |
| Brachycentridae: Brachycentrus       |       |     |     |      | 217   |     |      |      |      |      |     |     |     |
| fuliginosus                          | 5     | 5   |     |      | 326*  |     |      |      |      |      |     |     |     |
| : B. numerosus                       | 1     | 1   |     |      | 76    |     |      |      |      |      |     |     |     |
| Hydrophilidae: Macronemum zebratum   | 71    | 38  |     |      | 148** | 140 | 207  | 168  |      |      |     |     |     |
| Hydropsychidae: Hydropsyche          | 5     | 5   |     |      |       |     | 458* |      |      | 429* |     |     |     |
| : Parapsyche                         | 3     | 3   |     |      |       |     |      |      |      | 429  |     |     |     |
| Limnephilidae: Limnephilus           |       |     |     |      |       |     |      |      |      |      |     |     |     |
| consocius                            | 16    | 16  |     |      |       |     | 94   |      |      |      |     |     |     |
| : Limnephilus rhombicus              | 1     | 1   |     |      |       |     | 427* |      |      |      |     |     |     |
| Psychomyiidae: Polycentropus remotus | 1     | 1   |     |      |       |     | 485* |      |      |      |     |     |     |
| TOTAL                                | 679   | 493 |     |      |       |     |      |      |      |      |     |     |     |

\* without screen in respiratory flask; \*\* without screen in respiratory flask, multiple specimens;

\*\*\* multiple specimens.

TABLE 2. Average oxygen consumption (at 20 C in the Warburg apparatus; with screen in flask) and wet weight of individual immature aquatic insects.

| INSECT                                       | NO. SPECIMENS | AVERAGE WET WEIGHT(mg) | AVERAGE $\mu$ l O <sub>2</sub> /mg wet weight;hr |
|----------------------------------------------|---------------|------------------------|--------------------------------------------------|
| DIPTERA                                      |               |                        |                                                  |
| :RHAGIONIDAE: <u>Atherix variegata</u>       | 4             | 40.2 $\pm$ 8.6         | 145.00 $\pm$ 73.68                               |
| :TIPULIDAE: <u>Tipula</u>                    | 23            | 731.8 $\pm$ 551.4      | 46.80 $\pm$ 15.52                                |
| EPHEMEROPTERA                                |               |                        |                                                  |
| :BAETISIDAE: <u>Baetisca</u>                 | 16            | 25.7 $\pm$ 12.3        | 313.70 $\pm$ 82.89                               |
| :EPHEMERIDAE: <u>Ephemera</u>                | 8             | 12.5 $\pm$ 3.5         | 253.25 $\pm$ 34.64                               |
| :HEPTAGENIIDAE: <u>Stenonema</u>             | 6             | 22.6 $\pm$ 3.8         | 282.70 $\pm$ 114.36                              |
| :LEPTOPHEBIIDAE: <u>Leptophlebia</u>         | 16            | 32.6 $\pm$ 6.1         | 485.30 $\pm$ 86.89                               |
| :POLYMITARCIDAE: <u>Ephoron</u>              | 4             | 46.5 $\pm$ 15.9        | 307.00 $\pm$ 89.60                               |
| :POTAMANTHIDAE: <u>Potamanthus</u>           | 16            | 34.3 $\pm$ 15.4        | 293.60 $\pm$ 108.70                              |
| :SIPHONURIDAE: <u>Isonychia</u>              | 5             | 65.0 $\pm$ 19.8        | 440.00 $\pm$ 121.00                              |
| NEUROPTERA                                   |               |                        |                                                  |
| :CORYDALIDAE: <u>Chauliodes</u>              | 8             | 360.2 $\pm$ 152.6      | 67.30 $\pm$ 21.21                                |
| ODONATA                                      |               |                        |                                                  |
| :AESHNIDAE: <u>Aeshna verticalis</u>         | 5             | 477.1 $\pm$ 95.3       | 125.40 $\pm$ 52.72                               |
| :ANAXIDAE: <u>Anax junis</u>                 | 1             | 678.7                  | 125.00                                           |
| :GOMPHIDAE: <u>Gomphoides</u>                | 3             | 253.7 $\pm$ 175.4      | 99.00 $\pm$ 36.60                                |
| :GOMPHIDAE: <u>Gomphus</u>                   | 3             | 346.7 $\pm$ 39.2       | 102.00 $\pm$ 71.13                               |
| PLECOPTERA                                   |               |                        |                                                  |
| :PTERONARCIDAE: <u>Pteronarcys</u>           | 34            | 378.7 $\pm$ 41.2       | 87.20 $\pm$ 46.04                                |
| TRICHOPTERA                                  |               |                        |                                                  |
| :HYDROPSYCHIDAE: <u>Macronemum zebratum</u>  | 11            | 66.1 $\pm$ 18.4        | 207.00 $\pm$ 43.01                               |
| :LIMNephilidae: <u>Limnephilus consocius</u> | 16            | 126.8 $\pm$ 34.3       | 94.00 $\pm$ 56.66                                |

TABLE 3. Oxygen consumption rates on immature aquatic insects obtained by previous investigators by the Warburg and Barcroft methods.

| INSECT                               | HABITAT      | METHOD   | TEMP. | OXYGEN CONSUMPTION (MEAN) | REFERENCE                       |
|--------------------------------------|--------------|----------|-------|---------------------------|---------------------------------|
| <b>DIPTERA:</b>                      |              |          |       |                           |                                 |
| <i>Chironomus riparius</i> *         |              | Warburg  | 20 C  | 416                       | Edwards, 1958                   |
| <i>Simulium ornatum</i> (early pupa) |              | Warburg  | 25 C  | 2                         | Hinton, 1964                    |
| <i>Simulium ornatum</i> (mid pupa)   |              | Warburg  | 25 C  | 3.7                       | Hinton, 1964                    |
| <i>Simulium ornatum</i> (late pupa)  |              | Warburg  | 25 C  | 5.6                       | Hinton, 1964                    |
| <b>EPHEMEROPTERA:</b>                |              |          |       |                           |                                 |
| <i>Baetis rhodani</i> *              | Swift stream | Barcroft | 10 C  | 2571                      | Fox & Simmonds, 1933            |
| <i>Baetis rhodani</i> *              | Swift stream | Barcroft | 16 C  | 5051                      | Fox & Simmonds, 1933            |
| <i>Caenis</i> sp.*                   | Pond         | Barcroft | 10 C  | 290                       | Fox, Simmonds, Washbourn, 1935  |
| <i>Chloeon dipterum</i> *            | Pond         | Barcroft | 10 C  | 606                       | Fox, Simmonds, Washbourn, 1935  |
| <i>Chloeon dipterum</i> *            | Pond         | Barcroft | 16 C  | 1792                      | Fox & Simmonds, 1933            |
| <i>Ecdyonurus venosus</i>            | Swift stream | Barcroft | 10 C  | 604                       | Fox, Simmonds, Washbourn, 1935  |
| <i>Ecdyonurus venosus</i>            | Swift stream | Barcroft | 10 C  | 1321                      | Fox, Simmonds, Washbourn, 1935  |
| <i>Ephemera danica</i>               | Stream       | Barcroft | 10 C  | 370                       | Fox, Simmonds, Washbourn, 1935  |
| <i>Ephemera danica</i>               | Stream       | Barcroft | 10 C  | 829                       | Fox, Simmonds, Washbourn, 1935  |
| <i>Ephemera vulgata</i>              | Pond         | Barcroft | 10 C  | 278                       | Fox, Simmonds, Washbourn, 1935  |
| <i>Ephemera vulgata</i>              | Pond         | Barcroft | 10 C  | 740                       | Fox, Simmonds, Washbourn, 1935  |
| <i>Ephemerella ignita</i> *          | Stream       | Barcroft | 10 C  | 950                       | Fox, Simmonds, Washbourn, 1935  |
| <b>TRICHOPTERA:</b>                  |              |          |       |                           |                                 |
| <i>Cheumatopsyche comis</i> *        |              | Warburg  | 21 C  | 1030+50                   | Norris, Arnold, & Edwards, 1964 |
| <i>Cheumatopsyche comis</i> *        |              | Warburg  | 30 C  | 1500+40                   | Norris, Arnold, & Edwards, 1964 |
| <i>Chimarra betteni</i> *            |              | Warburg  | 21 C  | 825+74                    | Norris, Arnold, & Edwards, 1964 |
| <i>Chimarra betteni</i> *            |              | Warburg  | 30 C  | 875+25                    | Norris, Arnold, & Edwards, 1964 |
| <i>Hydropsyche</i> sp.*              |              | Barcroft | 10 C  | 770                       | Fox & Simmonds, 1933            |
| <i>Limnephilus vittatus</i> *        | Swift stream | Barcroft | 10 C  | 511                       | Fox & Simmonds, 1933            |
| <i>Notiomia ornata</i> *             | Pond         | Barcroft | 10 C  | 550+80                    | Norris, Arnold, & Edwards, 1964 |
| <i>Notiomia ornata</i> *             |              | Warburg  | 21 C  | 565+45                    | Norris, Arnold, & Edwards, 1964 |
|                                      |              | Warburg  | 30 C  |                           |                                 |

\*Multiple specimens used; \*\*anesthetized; \*\*\*larvae removed from their cases.

TABLE 4. Comparison of oxygen consumption rates on immature aquatic insects obtained with the electro-chemical flow-through method and the Warburg apparatus.

| ELECTRO-CHEMICAL FLOW-THROUGH METHOD |                    |             |                             |                               | WARBURG METHOD     |             |                                  |
|--------------------------------------|--------------------|-------------|-----------------------------|-------------------------------|--------------------|-------------|----------------------------------|
| SPECIMEN & EXPERIMENT<br>DATE(S)     | NO.<br>of<br>SPEC. | °C<br>TEMP. | FLOW-BY<br>RATE<br>IN./MIN. | μl O <sub>2</sub> :<br>gm:hr* | EXPERIMENT<br>DATE | °C<br>TEMP. | MEAN<br>μl O <sub>2</sub> :gm:hr |
| TRICHOPTERA:                         | 8                  | 23.6        | 1.66                        | 344                           | 27 IX 63           | 18          | 186                              |
| <u>Macronemum</u>                    | 8                  | 24.2        | 0.85                        | 264                           |                    |             |                                  |
| <u>zebratum</u>                      | 8                  | 24.9        | 0.45                        | 314                           |                    |             |                                  |
| 26 IX 63                             | 8                  | 24.7        | 0.23                        | 288                           |                    |             |                                  |
| PLECOPTERA:                          | 1                  | 22.1        | 0.465                       | 80                            | 10 III 64          | 13          | 60                               |
| <u>Pteronarcys</u>                   | 1                  | 21.3        | 0.232                       | 73                            |                    |             |                                  |
| <u>pictetii</u>                      | 1                  | 20.6        | 0.087                       | 85                            |                    |             |                                  |
| 12 III 64                            |                    |             |                             |                               |                    |             |                                  |
| PLECOPTERA:                          | 1                  | 20.6        | 0.460                       | 49                            | 10 III 64          | 13          | 29                               |
| <u>Pteronarcys</u>                   | 1                  | 21.4        | 0.232                       | 39                            | 24 III 64          | 19          | 39                               |
| <u>pictetii</u>                      | 1                  | 22.2        | 0.095                       | 55                            |                    |             |                                  |
| 13 III 64                            | 1                  | 19.6        | 1.147                       | 54                            |                    |             |                                  |
| 19 III 64                            | 1                  | 19.6        | 0.578                       | 24                            |                    |             |                                  |
|                                      | 1                  | 19.2        | 0.240                       | 19                            |                    |             |                                  |
| PLECOPTERA:                          | 1                  | 20.1        | 0.229                       | 127                           | 15 VI 64           | 20          | 100                              |
| <u>Pteronarcys</u>                   | 1                  | 19.8        | 0.286                       | 119                           |                    |             |                                  |
| <u>pictetii</u>                      | 1                  | 20.55       | 0.092                       | 115                           |                    |             |                                  |
| 10 VI 64                             | 1                  | 20.8        | 0.092                       | 114                           |                    |             |                                  |
| 11 VI 64                             | 1                  | 20.9        | 0.454                       | 129                           |                    |             |                                  |
|                                      | 1                  | 19.8        | 0.286                       | 119                           |                    |             |                                  |
| ODONATA:                             | 1                  | 21.8        | 0.467                       | 136                           | 9 I 64             | 20          | 149                              |
| <u>Aeshna</u>                        | 1                  | 22.5        | 0.240                       | 280                           | 14 I 64            | 20          | 172                              |
| <u>verticalis</u>                    | 1                  | 22.6        | 0.097                       | 215                           |                    |             |                                  |
| 2 I 64                               | 1                  | 23.0        | 1.173                       | 146                           |                    |             |                                  |
| 8 I 64                               | 1                  | 23.2        | 0.589                       | 100                           |                    |             |                                  |
|                                      | 1                  | 23.2        | 0.228                       | 98                            |                    |             |                                  |
| ODONATA:                             | 1                  | 21.4        | 0.473                       | 78                            | 16 IV 64           | 21          | 132                              |
| <u>Aeshna</u>                        | 1                  | 21.3        | 0.232                       | 82                            |                    |             |                                  |
| <u>verticalis</u>                    | 1                  | 21.4        | 0.0943                      | 60                            |                    |             |                                  |
| 31 III 64                            | 1                  | 22.3        | 1.147                       | 67                            |                    |             |                                  |
| 7 IV 64                              | 1                  | 21.7        | 0.582                       | 73                            |                    |             |                                  |
|                                      | 1                  | 22.6        | 0.237                       | 52                            |                    |             |                                  |
| ODONATA:                             | 1                  | 22.2        | 0.473                       | 198                           | 9 I 64             | 20          | 125                              |
| <u>Anax</u>                          | 1                  | 22.6        | 0.237                       | 193                           |                    |             |                                  |
| <u>junis</u>                         | 1                  | 23.0        | 0.097                       | 98                            |                    |             |                                  |
| 7 I 64                               | 1                  | 22.7        | 1.178                       | 103                           |                    |             |                                  |
| 15 I 64                              | 1                  | 23.0        | 0.593                       | 105                           |                    |             |                                  |
|                                      | 1                  | 23.0        | 0.229                       | 104                           |                    |             |                                  |

\* at 760 mm & 0C

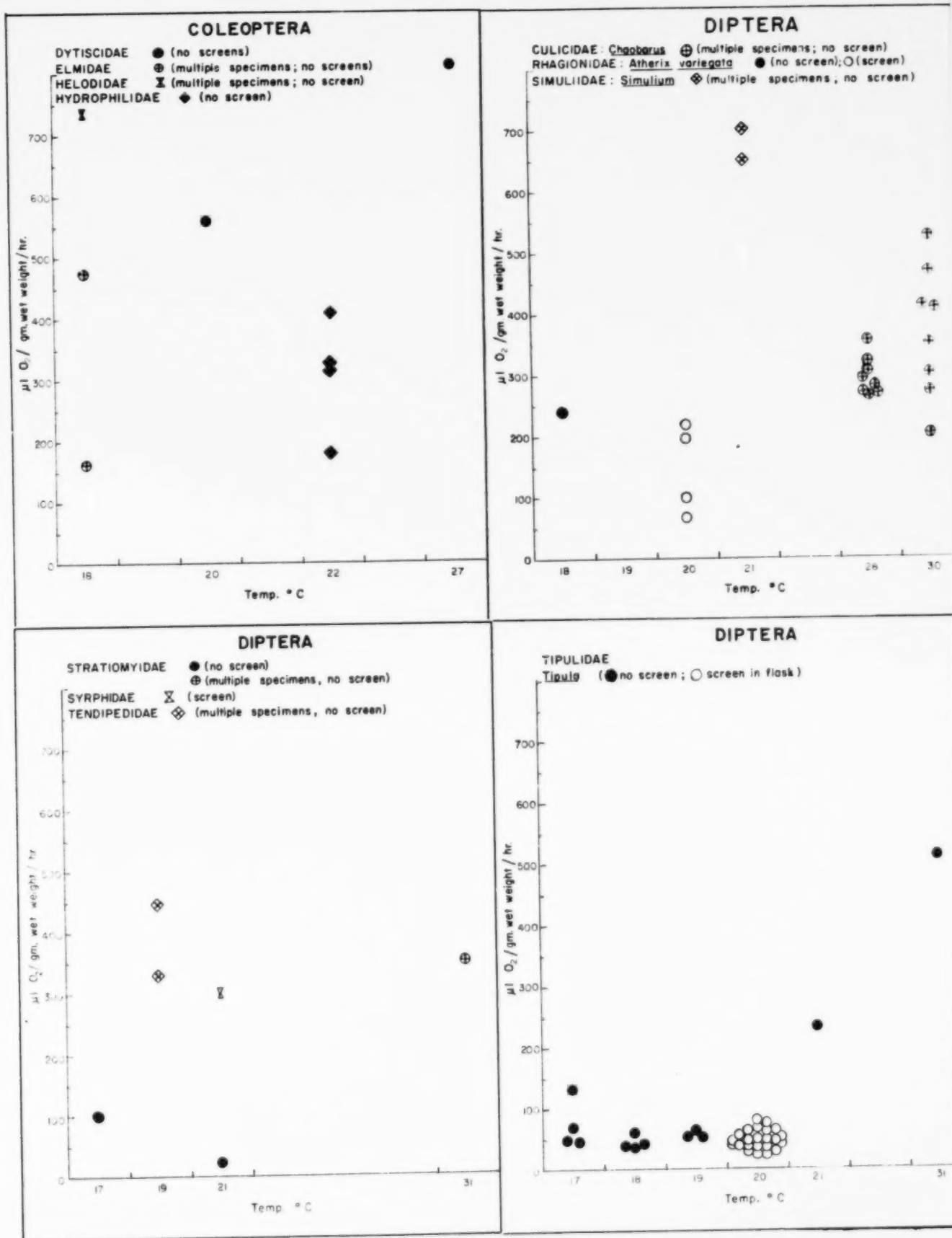


Fig. 1 Oxygen consumption of immature aquatic insects at various temperatures and conditions with the Warburg apparatus.

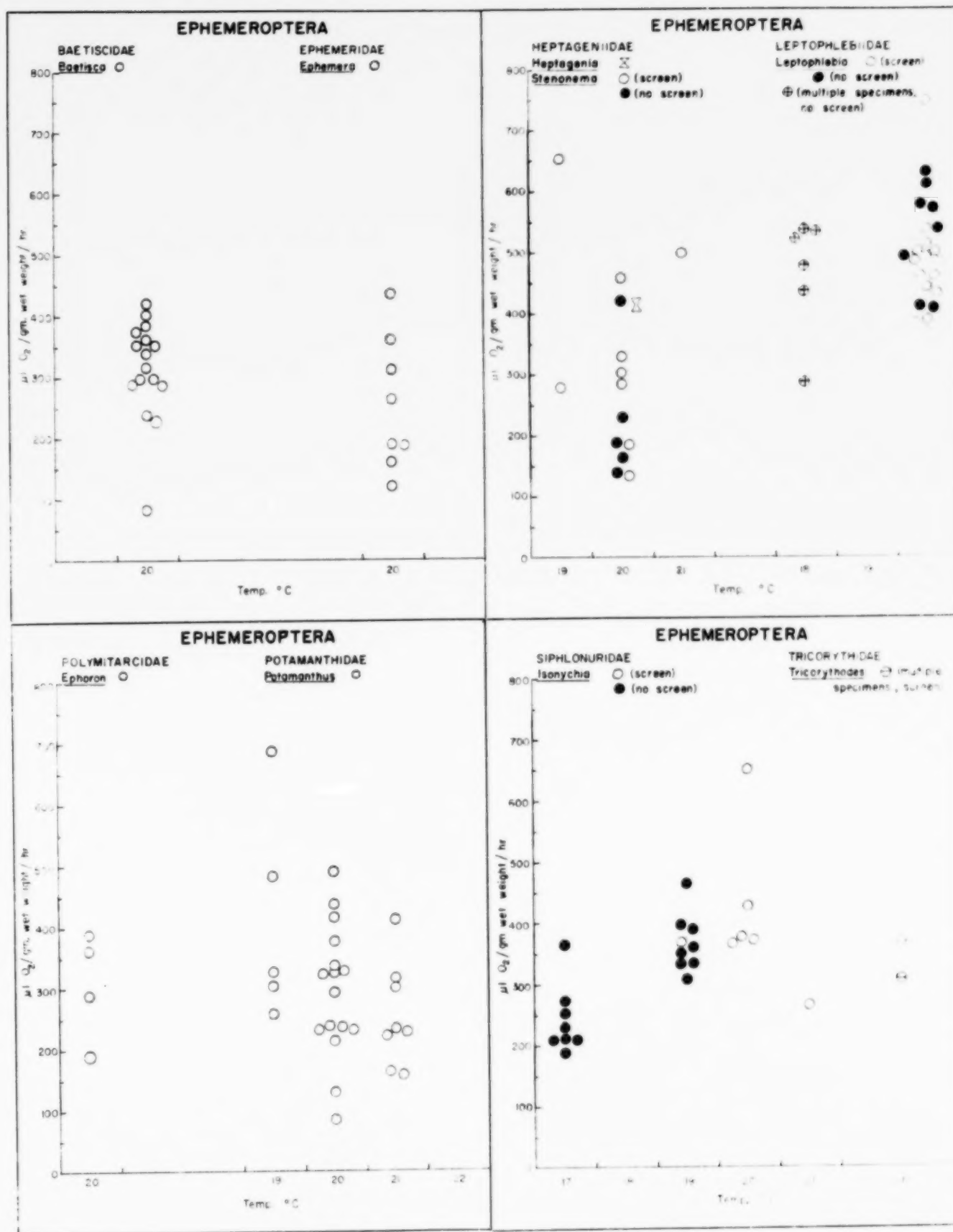


Fig. 2 Oxygen consumption of immature aquatic insects at various temperatures and conditions with the Warburg apparatus.

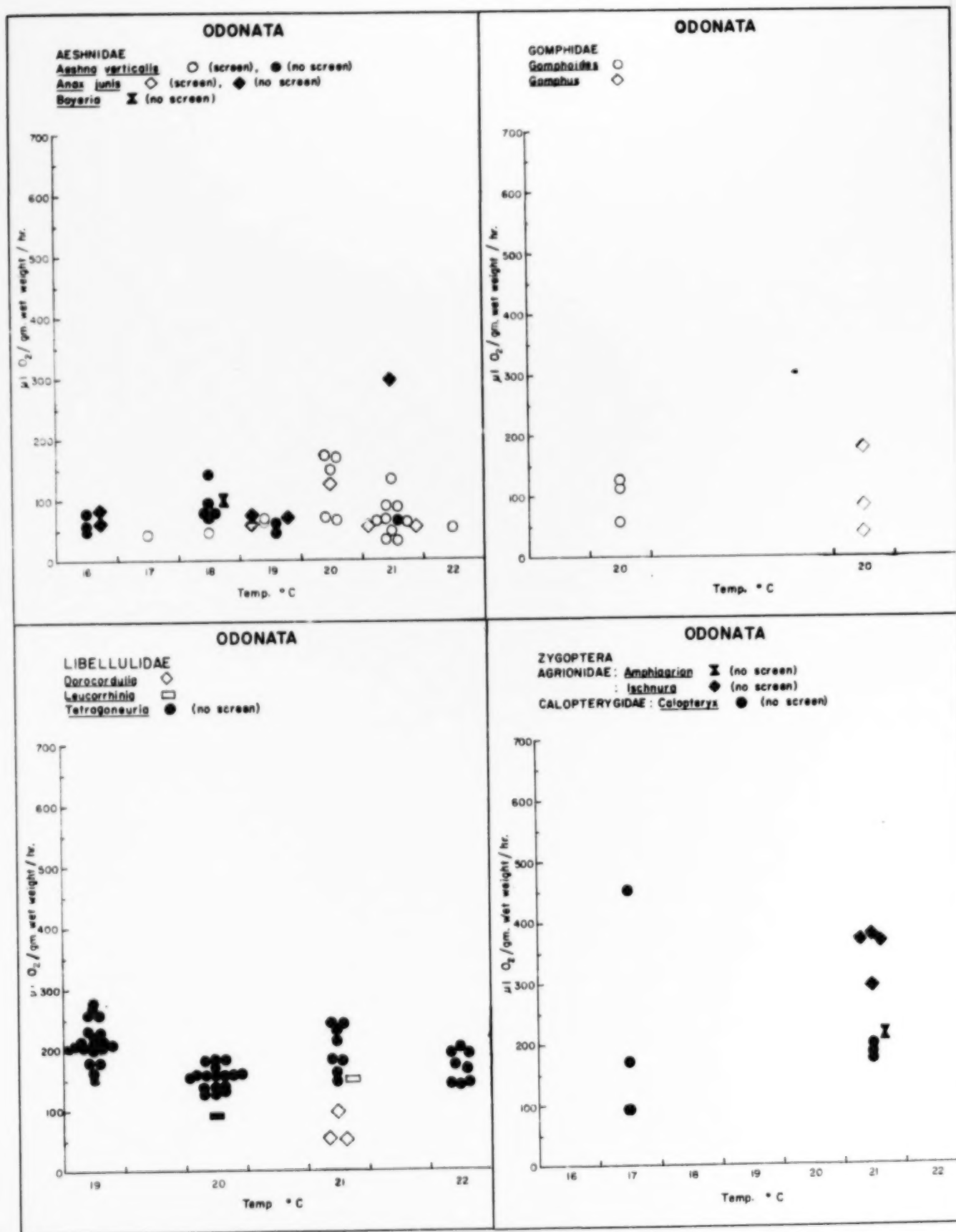


Fig. 3 Oxygen consumption of immature aquatic insects at various temperatures and conditions with the Warburg apparatus.

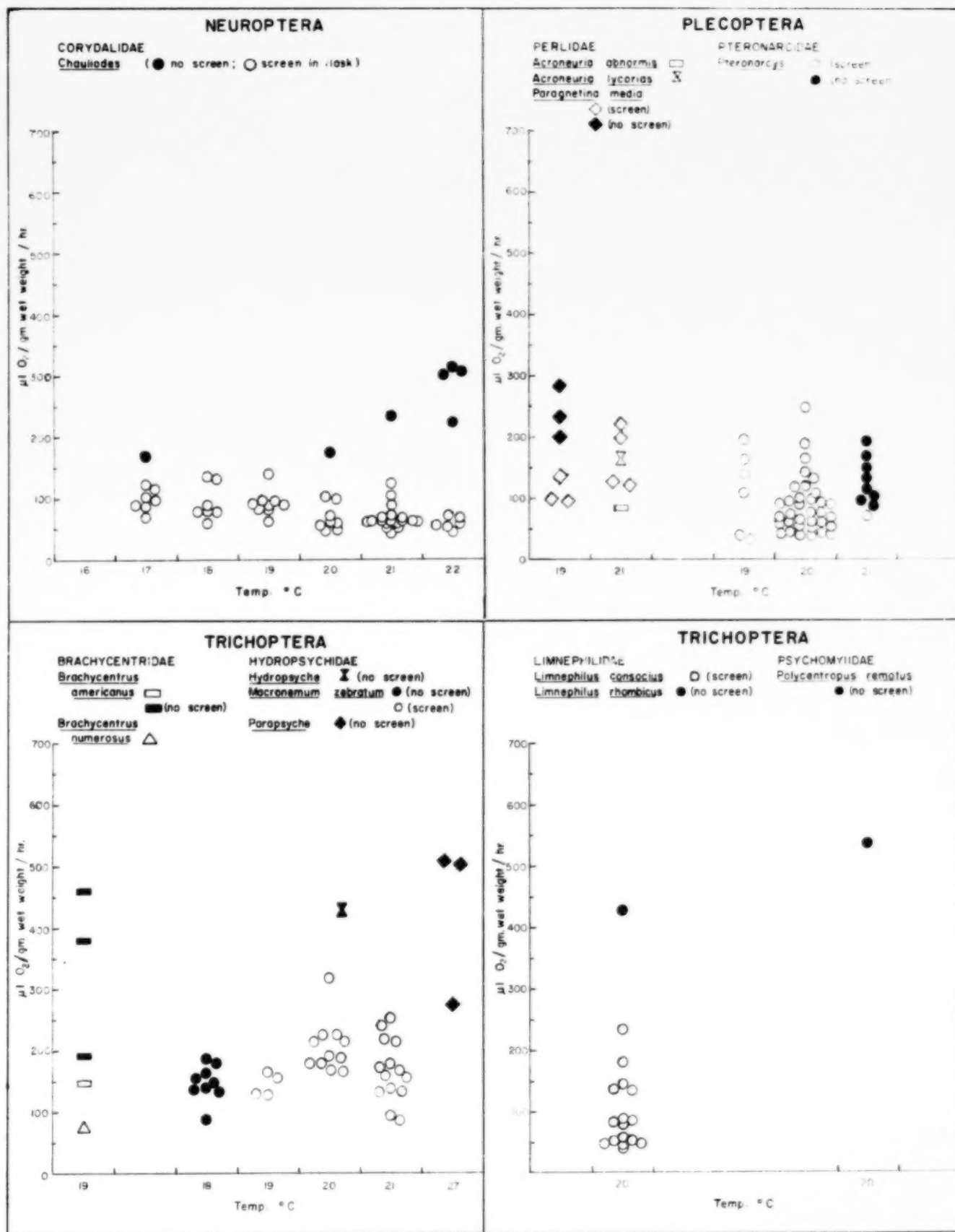


Fig. 4 Oxygen consumption of immature aquatic insects at various temperatures and conditions with the Warburg apparatus.

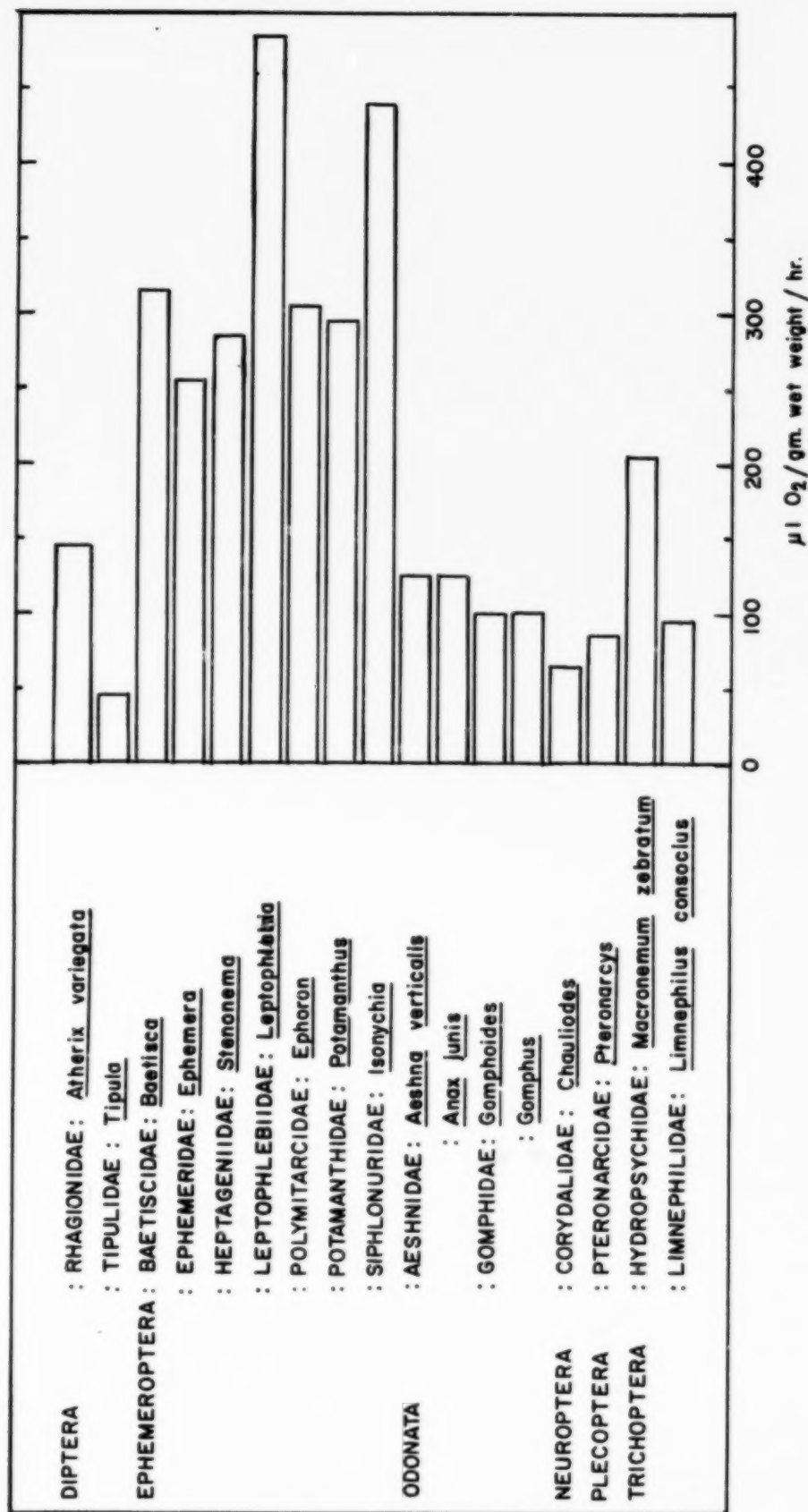


Fig. 5. Average oxygen consumption of individual immature aquatic insects at 20°C in the Warburg apparatus (with screen in flask).

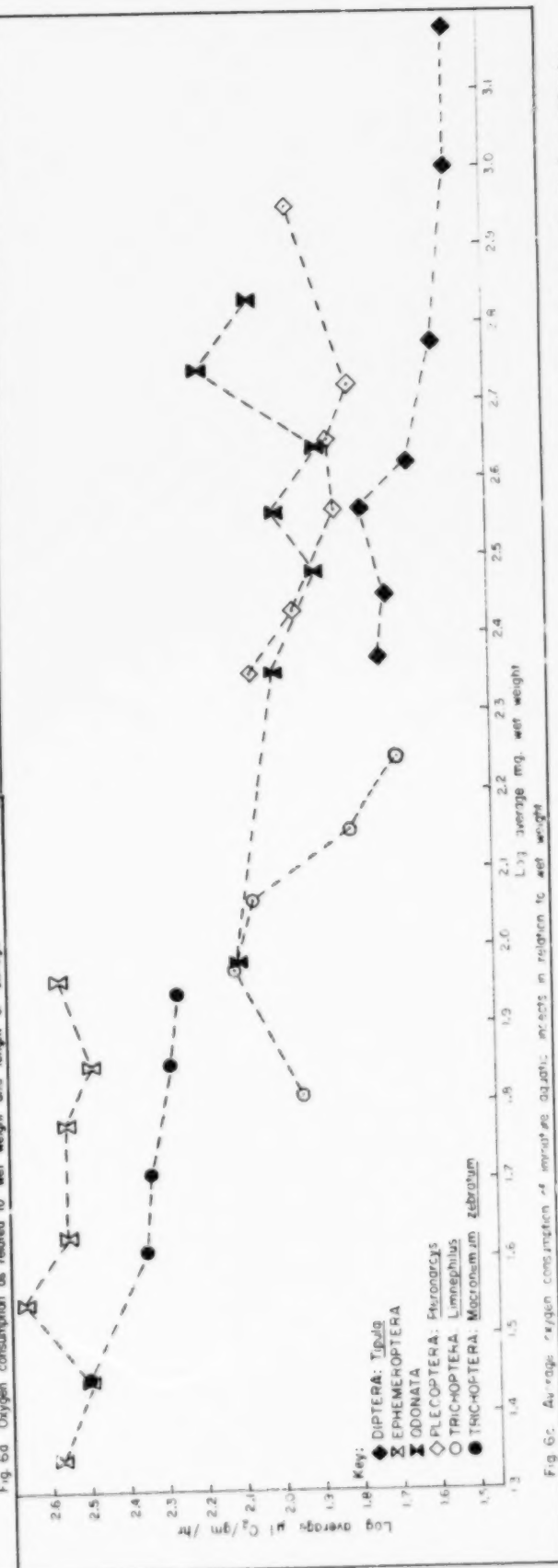
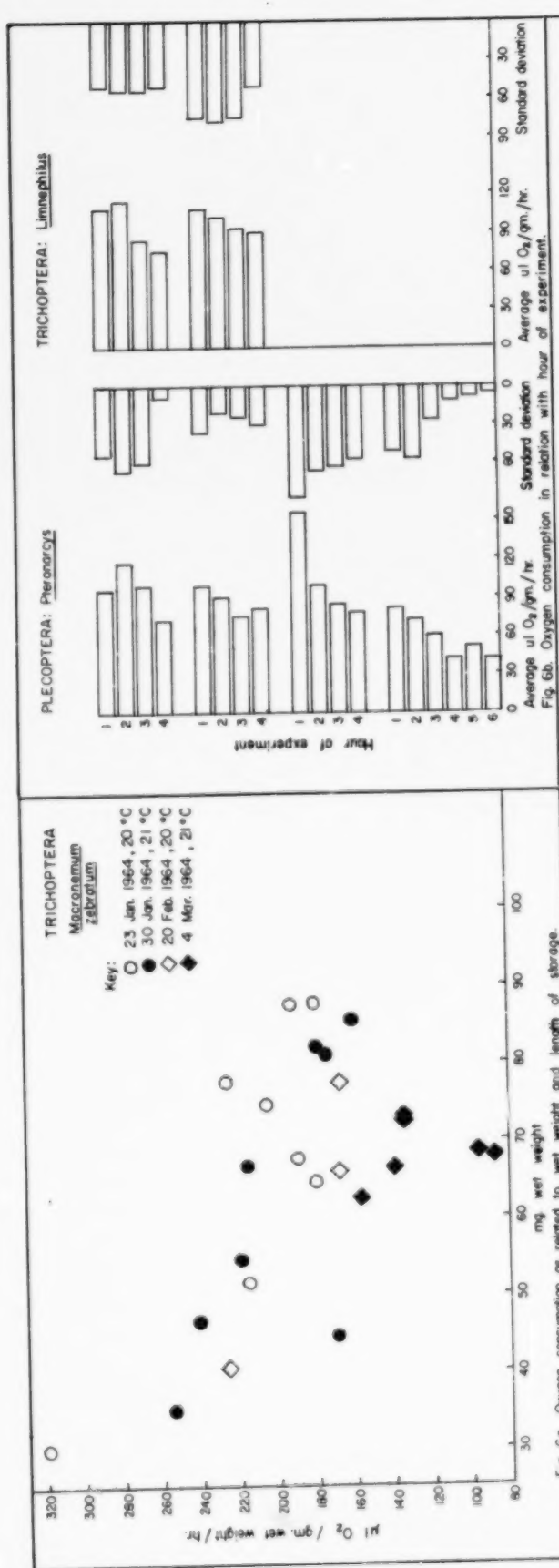


Fig. 6. Relationship of oxygen consumption of immature aquatic insects with wet weight, length of storage (in 5°C aquarium), hour of experiment, and insect group using the Warburg apparatus (screen in flask; 20°C unless otherwise indicated)

## F. BIBLIOGRAPHY

- Edwards, R. W., 1958. The relation of oxygen consumption to body size and to temperature in the larvae of Chironomus riparius Meigen. J. Exptl. Biol. 35:383-395.
- Edwards, R. W., and M. A. Learner, 1960. Some factors affecting the oxygen consumption of Asellus. J. Exptl. Biol. 37:706-718.
- Fair, G. M. and M. C. Whipple, 1948. Revision of The microscopy of drinking water by G. C. Whipple. 4th ed. John Wiley and Sons, Inc., New York. pp. 554-555.
- Fox, H. M. and B. G. Simmonds, 1933. Metabolic rates of aquatic arthropods from different habitats. J. Exptl. Biol. 10:67-74.
- Fox, H. M., B. G. Simmonds, and R. Washbourn, 1935. Metabolic rates of ephemerid nymphs from swiftly flowing and from still waters. J. Exptl. Biol. 12:179-184.
- Gaufin, A. R. and C. M. Tarzwell, 1952. Aquatic invertebrates as indicators of stream pollution. Public Health Reports (U.S.) 67:57-64.
- Heilbrunn, L. V., 1952. An outline of general physiology. 3rd ed. W. B. Saunders Co., Philadelphia. 818 pp.
- Hinton, H. E., 1964. The respiratory efficiency of the spiracular gill of Simulium. J. Insect Physiol. 10:73-80.
- Holman, H. H., 1962. Biological research method. Hafner Publish. Co., New York. 262 pp.
- Leech, H. B. and H. P. Chandler, 1956. Coleoptera. In Aquatic insects of California edited by R. L. Usinger. Univ. Calif. Press, Berkeley. pp. 293-371.
- Ludig, H. F., W. J. Oswald, and H. B. Gotaas, 1951. Manometric technique for measurement of BOD. Sanit. Eng. Lab., Univ. Calif., Inst. Eng. Research, Berkeley. 31 pp.
- Morgan, A. H. and H. D. O'Neil, 1931. The function of the tracheal gills in larvae of the caddis fly, Macronema zebratum Hagen. Physiol. Zool. 4:361-379.
- Norris, W. E., C. R. Arnold, and S. W. Edwards, 1964. Oxygen consumption by caddisfly larvae. Texas J. Sci. 16:72-79.
- Sinclair, R. M., 1964. Water quality requirements of the family Elmidae (Coleoptera) (with keys to the larvae and adults of the eastern genera). Tenn. Stream Pollut. Control Board, Tenn. Dept. Public Health. 14 pp.
- Smith, R. F. and A. E. Pritchard, 1956. Odonata. In Aquatic insects of California edited by R. L. Usinger. Univ. Calif. Press, Berkeley. pp. 106-153.
- Umbreit, W. W., R. H. Burris, and J. F. Stauffer, 1964. Manometric techniques. 4th ed. Burgess Publish. Co., Minneapolis. 305 pp.

Walshe, B. M., 1950. The function of haemoglobin in Chironomus plumosus under natural conditions. J. Exptl. Biol. 27:73-95.

Wigglesworth, V. B., 1950. The principles of insect physiology. E. P. Dutton and Co., Inc., New York. 544 pp.

References Used For The Identification Of Immature Aquatic Insects

Berner, L., 1950. The mayflies of Florida. Univ. Florida Studies, Biol. Sci. Series 4:1-267.

Burks, B. D., 1953. The mayflies, or Ephemeroptera, of Illinois. Bull. Illinois Nat. Hist. Sur. 26:1-216.

Claassen, P. W., 1931. Plecoptera nymphs of America (north of Mexico). Thos. Say Foundation of the Entomol. Soc. Am. Publ. No. 3:1-199.

Cuyler, R. D., 1958. The larvae of Chauliodes Latreille (Megaloptera:Corydalidae). Ann. Entomol. Soc. Am. 51:582-586.

Daggy, R. H., 1941. Taxonomic and biological investigations on Minnesota mayflies (Ephemeroptera). Doctor's Thesis, Univ. Minn. 356 pp.

Daggy, R. H., 1945. New species and previously undescribed naiads of some Minnesota mayflies (Ephemeroptera). Ann. Entomol. Soc. Am. 38:373-396.

Denning, D. G., 1937. The biology of some Minnesota Trichoptera. Trans. Am. Entomol. Soc. 63:17-43.

Denning, D. G., 1942. The Hydropsychidae of Minnesota (Trichoptera). Doctor's Thesis, Univ. Minn. 163 pp.

Edmundson, W. T. (Editor), 1959. Fresh-water biology. 2nd ed. John Wiley and Sons, Inc. 1248 pp.

Elkins, W. A., 1936. The immature stages of some Minnesota Trichoptera. Ann. Entomol. Soc. Am. 29:656-682.

Frison, T. H., 1935. The stoneflies, or Plecoptera, of Illinois. Bull. Illinois Nat. Hist. Survey 20:281-471.

Harden, P. H. and C. E. Mickel, 1952. The stoneflies of Minnesota (Plecoptera). Univ. Minn. Agr. Exp. Sta. Tech. Bull. No. 201:1-84.

Johannsen, O. A., 1934. Aquatic Diptera. Part I. Nemocera, exclusive of Chironomidae and Ceratopogonidae. Cornell Univ. Agr. Exp. Sta. Mem. 164:1-71.

Johannsen, O. A. 1935. Aquatic Diptera. Part II. Orthorrhapha-Brachycera and Cyclorrhapha. Cornell Univ. Agr. Exp. Sta. Mem. 177:1-62.

Johannsen, O. A. 1937. Aquatic Diptera. Part III. Chironomidae: subfamilies Tanypodinae, Diamesinae, and Orthocladiinae. Cornell Univ. Agr. Exp. Sta. Mem. 205:1-80.

Leonard, J. W. and F. A. Leonard, 1962. Mayflies of Michigan trout streams. Cranbrook Inst. of Science, Bloomfield, Mich. 139 pp.

- Lloyd, J. T., 1921. The biology of North American caddis fly larvae. Bull. Lloyd Library of Botany, Pharmacy and Materia Medica, Entomol. Series No. 1 21:1-124.
- Milne, M. J., 1939. Immature North American Trichoptera. Psyche 46:9-19.
- Needham, J. G. and M. J. Westfall, Jr., 1955. A manual of the dragonflies of North America (Anisoptera) including the Greater Antilles and the provinces of the Mexican border. Univ. Calif. Press, Berkeley and Los Angeles. 615 pp.
- Nicholson, H. P. and C. E. Mickel, 1950. The black flies of Minnesota (Simuliidae). Univ. Minn. Agr. Exp. Sta. Tech. Bull. 192:1-64.
- Parfin, S. I., 1952. The Megaloptera and Neuroptera of Minnesota. Am. Midland Naturalist 47:421-434.
- Pennak, R. W., 1953. Fresh-water invertebrates of the United States. Ronald Press Co., New York. 769 pp.
- Ross, H. H., 1944. The caddis flies, or Trichoptera, of Illinois. Bull. Illinois Nat. Hist. Survey 23:1-326.
- Sanderson, M. W., 1938. A monographic revision of the North American species of Stenelmis. Univ. Kansas Sci. Bull. 25:635-717.
- Sanderson, M. W., 1953. A revision of the Nearctic genera of Elmidae. Part I. J. Kansas Entomol. Soc. 26:148-163.
- Sanderson, M. W., 1954. A revision of the Nearctic genera of Elmidae. Part II. J. Kansas Entomol. Soc. 27:1-13.
- Usinger, R. L. (Editor), 1956. Aquatic insects of California, with keys to North American genera and California species. Univ. Calif. Press, Berkeley and Los Angeles. 508 pp.
- Walker, E. M., 1953. The Odonata of Canada and Alaska. Vol. 1, Part I, General; Part II, Zygoptera. Univ. Toronto Press, Toronto. 292 pp.
- Walker, E. M., 1958. The Odonata of Canada and Alaska. Vol 2, Part III; the Anisoptera -- four families. Univ. Toronto Press, Toronto. 318 pp.